Molecular Ecology (2007) **16**, 3866–3889 doi: 10.1111/j.1365-294X.2007.03453.x

Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems

DYLAN J. FRASER,* MICHAEL M. HANSEN,† SIRI ØSTERGAARD,† NATHALIE TESSIER,‡ MICHEL LEGAULT,§ LOUIS BERNATCHEZ¶

**Department of Biology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 1J1, †Technical University of Denmark, Danish Institute for Fisheries Research, Department of Inland Fisheries, Vejlsovej 39 DK8600, Silkeborg, Denmark, ‡Department de Sciences et de Biologie, Université de Montréal, Québec, Canada H3C 3J7, §Societé de la faune et des parcs du Québec, Direction de la recherche sur la faune, 675 boulevard René Lévesque Est, Québec, Canada G1R 5V7, ¶Departmente de Biologie, Université Laval, Sainte-Foy, Québec, Canada G1K 7P4*

Abstract

Estimation of effective population sizes (N_e) and temporal gene flow (N_em, m) has many implications for understanding population structure in evolutionary and conservation biology. However, comparative studies that gauge the relative performance of N_e, N_em or m methods are few. Using temporal genetic data from two salmonid fish population systems with disparate population structure, we (i) evaluated the congruence in estimates and precision of long- and short-term N_e, N_em and m from six methods; (ii) explored the effects of metapopulation structure on N_e estimation in one system with spatiotemporally linked subpopulations, using three approaches; and (iii) determined to what degree interpopulation gene flow was asymmetric over time. We found that long-term N_e estimates exceeded short-term N_e within populations by 2–10 times; the two were correlated in the system with temporally stable structure (Atlantic salmon, *Salmo salar*) but not in the highly dynamic system (brown trout, *Salmo trutta*). Four temporal methods yielded short-term N_e estimates within populations that were strongly correlated, and these were higher but more variable within salmon populations than within trout populations. In trout populations, however, these short-term N_e estimates were always lower when assuming gene flow than when assuming no gene flow. Linkage disequilibrium data generally yielded short-term N_e estimates of the same magnitude as temporal methods in both systems, but the two were uncorrelated. Correlations between long- and short-term geneflow estimates were inconsistent between methods, and their relative size varied up to eightfold within systems. While asymmetries in gene flow were common in both systems (58–63% of population-pair comparisons), they were only temporally stable in direction within certain salmon population pairs, suggesting that gene flow between particular populations is often intermittent and/or variable. Exploratory metapopulation N_e analyses in trout demonstrated both the importance of spatial scale in estimating N_e and the role of gene flow in maintaining genetic variability within subpopulations. Collectively, our results illustrate the utility of comparatively applying N_e, N_em and m to (i) tease apart processes implicated in population structure, (ii) assess the degree of continuity in patterns of connectivity between population pairs and (iii) gauge the relative performance of different approaches, such as the influence of population subdivision and gene flow on N_e estimation. They further reiterate the importance of temporal sampling replication in population genetics, the value of interpreting N_e or m in light of species biology, and the need to address long-standing assumptions of current N_e, N_em or m models more explicitly in future research.

Keywords: adaptive divergence, asymmetric dispersal, asymmetric gene flow, connectivity, effective population size, gene flow, genetic monitoring, linkage disequilibrium, local adaptation, metapopulation, migration rate, salmon, sink, temporal stability, trout

Received 2 March 2007; revision accepted 11 June 2007

Correspondence: Dylan J. Fraser, Fax: (902) 494-3736; E-mail: dylan.fraser@dal.ca

Introduction

A common thread linking molecular ecology research is the elucidation of patterns and processes underlying population genetic structure within species. Whether such structure is temporally stable or unstable is increasingly addressed for its utility in distinguishing how many populations exist as well as their inter-relationships (Lessios *et al*. 1994; Nielsen *et al*. 1999; Ruzzante *et al*. 2001; Hoffman *et al*. 2004; Dannewitz *et al*. 2005; Waples & Gaggiotti 2006). Insights gained from this research are in turn enlightening management decisions regarding, but not limited to, the definition of conservation units and genetic monitoring (Crandall *et al*. 2000; Fraser & Bernatchez 2001; Larsen *et al*. 2005; Hansen *et al*. 2006a; Palsboll *et al*. 2007; Schwartz *et al*. 2007).

A vital part of further understanding patterns of population genetic variation will come from integrating knowledge of effective population sizes (N_e) and temporal gene flow (migration rate, m). The rate of loss of genetic diversity via genetic drift is greater in populations when N_e is small and, in the absence of m, this rate is expected to increase as N_e decreases (Frankham *et al*. 2002). Losses of genetic diversity from drift can thus be counterbalanced by m (Lenormand 2002). Temporal gene flow can also reveal *continual* patterns of connectivity between populations, whether human or naturally-induced (Vasemagi *et al*. 2005; Fraser *et al*. 2007). In addition, the interplay between N_e and m can provide insight into the extent to which gene flow constrains local adaptation (Adkison 1995; Hendry *et al*. 2001; Hansen *et al*. 2002). Overall, because commonly used measures of population genetic differentiation only consider the product N_em [e.g. $F_{ST} = 1/(4N_em + 1)$], N_e and m studies illustrate that such measures often do not adequately portray the processes underlying genetic structure. Similar F_{ST} values, for example, might reflect both temporally stable populations with low gene flow and large N_e, and unstable populations with high gene flow and low N_e (Whitlock & McCauley 1999; Jensen *et al*. 2005).

Recent developments have improved the feasibility of estimating N_e and temporal gene flow in natural populations (Beerli & Felsenstein 2001; Wilson & Rannala 2003; Wang 2005, Leberg 2005, and references therein). These methods account for common features of natural populations, including fluctuating population sizes and asymmetric gene flow, and thus have attracted considerable application (Turner *et al*. 2002; Ardren & Kapuscinski 2003; Miller & Waits 2003; Shrimpton & Heath 2003; Fraser *et al*. 2004, 2007; Hoffman *et al*. 2004; Johnson *et al*. 2004; Kaeuffer *et al*. 2004; Wilson *et al*. 2004; Consuegra *et al*. 2005; Jehle *et al*. 2005; Aspi *et al*. 2006; Hansen *et al*. 2006b, 2007; Poulsen *et al*. 2006; Weetman *et al*. 2006; Watts *et al*. 2007). Much of this research, however, was not conducted in a strictly comparative sense. Few simulation studies have also evaluated the precision, accuracy or computational efficiency of most N_e and m methods, and these have found that no estimator performed universally better than others across different simulations (e.g. with varying sample sizes, numbers of loci screened; Tallmon *et al*. 2004; see also Abdo *et al*. 2004; Araki *et al*. 2007; Faubet *et al*. 2007). Some assumptions likely violated in most natural systems have also not been evaluated thoroughly; namely, that populations are closed to migration when estimating N_e. Thus, as details about population size and connectivity are typically lacking a priori, it is currently difficult to know which N_e and m methods are most suitable to apply.

Challenges clearly arise when interpreting results from any comparative study (experimental or natural population data), since true N_e and m values are often unknown, and parameters affecting N_e and m cannot all be controlled for (Leberg 2005; Wang 2005). Nevertheless, an empirical comparison of several methods across a range of biological conditions would be useful to gauge their relative performance. For instance, discrepancies between methods in different environments might reveal untenable assumptions within the studied populations.

Here, we use microsatellite DNA data to compare several genetic methods for estimating N_e and m among populations of two salmonid fish species with similar life histories. The two population systems we consider are useful for such a comparison. They have well-established population structure from previous works and, broadly speaking, experience contrasting environmental conditions; one system [landlocked (freshwater) sympatric populations of Atlantic salmon, *Salmo salar*] is very temporally stable (Tessier & Bernatchez 1999) while the other (anadromous brown trout populations, *Salmo trutta*, from a Danish island) is highly dynamic (Østergaard *et al*. 2003) (see Materials & Methods). Such contrasting environments should have very different impacts on the estimation of N_e and m, and on the evaluation of population genetic structure (Whitlock & McCauley 1999; Leberg 2005; Wang 2005). Other attributes of the data sets for each system include similar genotypic output (seven microsatellite loci), mean heterozygosities (0.61–0.66), allelic richness (five to seven alleles/locus) and mean population sample sizes (40–50). However, the two systems have different global levels of genetic differentiation [salmon mean $F_{ST} = 0.109$ (95% CI 0.066–0.122) vs. trout mean $F_{ST} = 0.031$ (95% CI 0.016–0.053); 1990s values]. Each data set also includes most or all sources of immigrants and thus potential gene flow (see Beerli 2004a,b; Slatkin 2005), as well as temporal replicates of population samples generally spaced at least three generations apart, but not exceeding 15 generations. These are useful for applying temporal approaches to estimating N_e and for determining whether continual patterns of gene flow exist between populations. Finally, general trends in abundance during the time periods sampled for both systems did not suggest overly large N_e

values (< 200–300). At larger N_e, genetic estimates of N_e are known to have poorer precision regardless if the number of samples or loci is increased (Nei & Tajima 1981; Waples 1989; Luikart *et al*. 1999; Waples 2002a).

Our comparison of N_e methods focused primarily on short-term N_e using temporal and linkage disequilibrium approaches, as well as long-term N_e, using coalescent methods, because (i) our data sets were conducive to making such comparisons based on the inclusion of good temporal replicates, and (ii) these methods have also attracted the most theoretical and empirical attention in the recent literature (see Leberg 2005; Wang 2005; Waples 2006). We acknowledge, however, that current estimators of N_e assume no subpopulation structure, such that N_e estimates are biased if applied to a subpopulation that has considerable genetic connection to other subpopulations within a larger metapopulation (Hedrick & Gilpin 1997; Waples 2002a). Therefore, as an additional objective, we complemented N_e and m comparisons by considering approaches to address the potential effects of subpopulation structure on N_e (e.g. metapopulation structure) in the data set from a highly dynamic system.

Materials and methods

Population systems, species biology, and data

Sample data (seven unlinked microsatellite loci) originate from landlocked (freshwater) sympatric populations of Atlantic salmon in Lac-Saint Jean, Canada (Tessier & Bernatchez 1999) and anadromous brown trout populations inhabiting Bornholm Island, Baltic Sea, Denmark (Østergaard *et al*. 2003) (Fig. 1; Table 1; Appendix I). Genetic estimates of N_e and m were never conducted on the Lac Saint-Jean system (Tessier *et al*. 1997; Tessier & Bernatchez 1999), but we include estimates from two methods ('Methods 2 and 4' in this study) from Østergaard *et al*. (2003) in our comparisons below.

In both systems, rivers are used for spawning and as juvenile habitat for migratory salmon or trout (Fig. 1). Juveniles spend anywhere from 1–3 years within rivers before migrating to lake or ocean feeding areas, respectively. After an additional 2–4 years, sexually mature salmon and trout return to rivers to complete their lifecycle. Individuals of both species predominantly return to their natal rivers to spawn, but tagging data (Larsen 1970) suggest higher/stronger dispersal (straying) in Bornholm trout than in populations elsewhere. This likely relates to the unstable nature of Bornholm Rivers (Østergaard *et al*. 2003). While 42 rivers exist on the island, most (≈ 25/42) are devoid of trout, and those inhabited are shallow, short (< 10 km) and highly sensitive to low precipitation. During periods of drought, rivers can dry up, leading to extinction of juvenile trout. Recolonization has been observed,

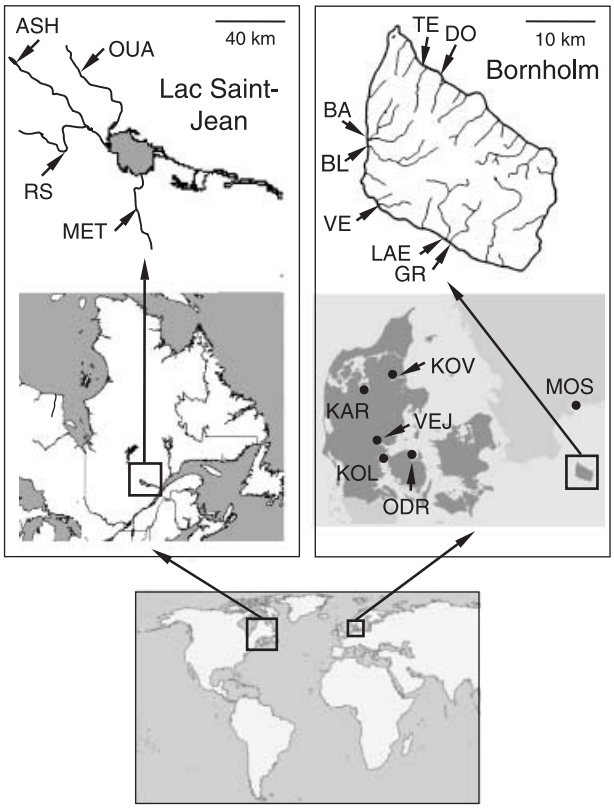

Fig. 1 Geographic locations of Lac-Saint Jean (Québec, Canada) and the island of Bornholm (Denmark). Abbreviations of river names show the location of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) populations used in this study. Full river names are found in Table 1.

and this system is considered an example of a natural metapopulation with high population turnover (Østergaard *et al*. 2003). Accordingly, Østergaard *et al*. (2003) observed dramatic changes in population genetic structure of Bornholm trout populations from the 1940s to the 1990s, reflected by considerable allelic frequency change and greater temporal differentiation within rivers than spatial differentiation between rivers. Other anadromous trout populations are found nearby just north of Bornholm, notably the large Mörrumsån River. Tagged trout from rivers to the west are also caught during feeding migrations close to Bornholm, so we included samples from these regions as potential gene flow sources for certain analyses (see Table 1). The four rivers entering Lac Saint-Jean and used by salmon are conversely larger (> 30–100 km) with more stable flow and environmental regimes (M. Legault, unpublished data). Correspondingly, salmon population structure in Lac Saint-Jean has been very temporally stable from the 1970s to 1990s, as reflected by similar allelic frequency distributions and F_{ST} estimates among populations (and between temporal periods within populations) (Tessier & Bernatchez 1999).

Table 1 Origins and numbers of samples for estimating effective population sizes and temporal gene flow in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) populations. Geographic locations of populations are found in Figure 1

Population	Population code	Years sampled (*n*)	Reference
S. salar: Lac Saint-Jean			
Riviere Aux Saumons	RS	1970 (40); 1994 (37)	Tessier & Bernatchez (1999)
Riviere Ashuapmushuan	ASH	1978 (39); 1994 (43)	Tessier & Bernatchez (1999)
Riviere Ouasiemsca	OUA	1980 (37); 1994 (36)	Tessier & Bernatchez (1999)
Riviere Metabetchouane	MET	1981 (38); 1994 (42)	Tessier & Bernatchez (1999)
S. trutta: Bornholm			
Vellens River	VE	1950 (49); 1992 (22); 1997 (50)	Østergaard *et al*. (2003)
Blykobbe River	BL	1944 (45); 1950 (46); 1997 (50)	Østergaard *et al*. (2003)
Dondals River	DO	1966 (29); 1992 (18); 1997 (50)	Østergaard *et al*. (2003)
Tejn River	TE	1944 (41); 1951 (47); 1992 (23); 1997 (50)	Østergaard *et al*. (2003)
Grodeby River	GR	1997 (40)	Østergaard *et al*. (2003)
Laesaa River	LAE	1997 (50)	Østergaard *et al*. (2003)
Baggeaa River	BA	1997 (40)	Østergaard *et al*. (2003)
S. trutta: Potential Sources to Bornholm			
Morrumsan River	MOS	1998 (50)	Fritzner *et al*. (2001)
Vejle River	VEJ	1910 (40); 1998 (50)	Hansen *et al*. (2002)
Karup River	KAR	1912 (47); 1951 (68); 1993 (49); 1996 (72)	Hansen *et al*. (2002)
Kovads River	KOV	1953 (36); 1996 (50)	Hansen *et al*. (2002)
Odder River	ODR	1998 (40)	Hansen *et al*. (2002)
Kolding River	KOL	1998 (50)	Hansen *et al*. (2002)

Short-term effective population size (N_e) estimates from the temporal method

For both data sets, we employed four 'temporal' methods with differing mathematical properties to estimate N_e in each population, based on short-term allelic frequency changes between sampling periods (Lac Saint Jean: circa 1980–1994; Bornholm circa 1950–1997). Methods 1–3 implemented (1) Waples' (1989) classic moment estimator, (2) Wang's (2001) pseudo-likelihood method and (3) Beaumont's (2003) maximum-likelihood, coalescent-based method. These methods chiefly assumed that mutation, selection and migration were unimportant in changing population allelic frequencies relative to genetic drift. Method 4 (Wang & Whitlock 2003) differed from Methods 1–3 in relaxing the assumption of no migration, so we hereafter distinguish N_e estimates from the two types of temporal methods as '$N_{e\mathrm{CLOSED}}$' (Methods 1–3) and '$N_{e\mathrm{OPEN}}$' (Method 4). We now briefly consider the importance of these and other key assumptions of temporal methods.

No mutation. This assumption is likely met because sampling periods in our study (maximum 15 generations) are sufficiently short that the effects of mutations can be safely ignored (Waples 1989; Beaumont 2003).

No selection. This assumption is more ambiguous and perhaps more difficult to ignore. Selection can cause higher or lower allelic frequency change than under the expectation of pure genetic drift. It could particularly bias N_e downwards if it is variable (Mueller *et al*. 1985), as genes or alleles associated within parents may be selected against in offspring (i.e. later generation) samples, leading to more genetic change in the population than under drift alone (Araki *et al*. 2007). Unfortunately, the modest number of loci utilized in our study prevented any rigorous statistical inferences from tests aimed at detecting potential selection at 'outlier' loci (Campbell & Bernatchez 2004; Guinand *et al*. 2004), but we consider this assumption further in parts of the Discussion.

No migration. For Methods 1–3, $N_{e\mathrm{CLOSED}}$ will be biased if effects of migration on allele frequencies are large relative to the effect of genetic drift (Wang & Whitlock 2003; see also Vitalis & Couvet 2001). Wang & Whitlock (2003) have argued that over few generations, migration increases allele frequency change compared to change due to drift alone, thereby causing underestimation of N_e. In contrast, the authors suggested that over many generations, migration may act as a buffer by maintaining genetic diversity that would otherwise have been lost by drift, leading to an overestimation of N_e. These points are somewhat simplistic because they are only true for particular sorts of migration. For instance, in the short-term, migration from a genetically similar source would not necessarily increase allele frequency change relative to drift alone. Nevertheless, the acknowledgement that migration can affect N_e estimation was pertinent to consider in

both Lac Saint-Jean and Bornholm, as a priori, we knew that gene flow was likely considerable between certain populations, especially in Bornholm. Both Nei & Tajima (1981) and Waples (1989, 2002a) also point out that where migration occurs, it is important to consider whether samples have been drawn from entire populations or subunits of populations. In Lac Saint-Jean salmon, subpopulation structure is not evident within rivers (Tessier *et al*. 1997; Tessier & Bernatchez 1999), but trout found in different rivers on the island of Bornholm may be interconnected as part of a larger metapopulation (Østergaard *et al*. 2003; see below).

Discrete generations. This assumption is violated since salmon and trout have overlapping generations. Our use of these methods thus assumed that individual cohorts had been sampled representatively with respect to age from the entire population. If not, the bias in N_e estimation will depend on how much genetic relationships among cohorts co-vary (Jorde & Ryman 1996). Nevertheless, Waples & Yokota (2007) have recently shown that this bias is minimized by taking samples separated by a minimum of three to five generations. In seven of eight populations in our study, temporal samples meet these requirements.

Note that Methods 1–4 also assumed random sampling within populations. Sampling plan II (see Nei & Tajima 1981; Waples 1989) was employed in the 'early' samples of both systems (pre-1990s) wherein individuals were sampled before reproduction and not replaced (post-1990 samples followed sampling plan I wherein fish were sampled nonlethally). These sampling characteristics mean that allele frequencies in our temporal samples are mutually exclusive within populations and can be considered as independent binomial draws, and so population size (N) is not a factor in affecting N_e estimation with the temporal method (see Waples 1989). Methods 1–4 also required accurate data on numbers of generations (T) between sampling periods, which we calculated for each salmon population using long-term mean spawning age data. Generation times for salmon populations (the number of aged individuals in parentheses) were as follows: RS = 5.54 years (n = 1966); ASH = 5.44 years (n = 611); OUA = 5.25 years (n = 4113); MET = 6.08 years (n = 552). Generation time was not available for Bornholm trout, but for anadromous trout in nearby regions it is 3.8 years; we used 3.5 years to account for the possibility of precocious males spawning at 2 years, following Østergaard *et al*. (2003).

Method 1. We calculated moment estimates of $N_{e\text{CLOSED}}$ and their 95% confidence intervals (CI) according to equations of Waples (1989) in Microsoft Excel. Method 1 can underestimate $N_{e\text{CLOSED}}$ when there are a number of low-frequency alleles in the data (Waples 1990a). Nevertheless, revised data sets for both population systems that pooled low-frequency alleles at each locus into one allelic class (< 0.02 over both sampling periods, as suggested by Waples 1990a) resulted in little or no change in $N_{e\text{CLOSED}}$ estimates compared to full data sets containing rarer alleles. Consequently, only $N_{e\text{CLOSED}}$ estimates from full data sets are presented. Method 1 also did not account for the potential influence of salmonid population differences in age structure on within-population N_e estimation in either system. However, we did not employ an analogous model that accounts for this possible effect because the model assumes semelparity (i.e. it is based on the Pacific salmon life history: see Waples 1990a, b) whereas brown trout and Atlantic salmon are iteroparous, and at least the degree to which Lac Saint-Jean Atlantic salmon are iteroparous most likely invalidates the model (M. Legault, unpublished data).

Method 2. We used the program MLNE 2.3 (Wang & Whitlock 2003) to estimate $N_{e\text{CLOSED}}$ according to Wang's (2001) pseudo-likelihood method. When running MLNE, we applied a maximum $N_{e\text{CLOSED}}$ of 1000. Upper 95% CI approaching 1000 were assumed to be infinity. Pseudo-likelihood methods only allow integers for sampling intervals; we carried out analyses with the following T' values and then $N_{e\text{CLOSED}}$ estimates ($N_{e\text{CLOSED}}'$) were converted by $N_{e\text{CLOSED}} = (T/T')\ N_{e\text{CLOSED}}'$: salmon RS = 4; ASH = 3, OUA = 3; MET = 2); trout (VE = 13; BL = 15; DO = 9; TE = 15). Simulations have shown more accuracy and precision in pseudo-likelihood or likelihood methods than moment estimators — the former use more information about the data in relation to sample sizes and T, whereas the latter can overestimate $N_{e\text{CLOSED}}$ when genetic drift is strong (Tallmon *et al*. 2004).

Method 3. Beaumont's (2003) coalescence-based likelihood method uses Markov chain Monte Carlo (MCMC) simulations to generate a posterior distribution of $N_{e\text{CLOSED}}$ over the entire sampling interval and incorporates a Bayesian prior on the maximum N_e ($N_{e\text{MAX}}$) to be set by the user. To evaluate the potential influence of prior information on the posterior distribution of $N_{e\text{CLOSED}}$, we set $N_{e\text{MAX}}$ at 1000 and 500 in each population and used 50 000 MCMC replicates.

Method 4. Method 4 jointly estimated $N_{e\text{OPEN}}$ and the generational migration rate, m, for the population in consideration within the time interval sampled (maximum $N_{e\text{OPEN}}$ applied = 1000); it thus required allele frequency data on the source population(s) contributing immigration to the population in question (Wang & Whitlock 2003). A major assumption of Method 4 is that populations receive constant migration from an infinite source of fixed allele frequency (Wang & Whitlock 2003). If potential sources

have not been sampled, or if sources change over time, this might lead to biases in N_{eOPEN} within the population receiving migration. For Lac Saint-Jean, we could assume that sampled populations were the only sources of migrants for each population, as barriers to the lake prevent mixture with outside populations. These populations also exhibited little allele frequency change between 1980 and 1994 (Tessier & Bernatchez 1999), meaning the assumption of stable allele frequencies in the source was roughly applicable. We thus pooled allele frequencies in the three other populations (from both sampling periods) to represent the allele frequencies of the source. However, RS, ASH and OUA likely exchange more gene flow with each other than with MET because of their closer genetic relationships (Tessier & Bernatchez 1999). For these three populations, we therefore also pooled allele frequencies from only the other two populations as the source to evaluate its effects on N_{eOPEN} estimation in each of these three populations. For Bornholm, Østergaard *et al*. (2003) estimated N_{eOPEN} in VE, BL, DO and TE using a similar approach to pooling allele frequencies into an 'infinite' source population. As considerable allelic change occurred within these populations between 1950 and 1997, the assumption of an infinite source of fixed allele frequency might affect N_{eOPEN} estimates. We treat this issue further below in relation to the effective metapopulation size of trout on Bornholm.

Short-term effective population size (N_e) estimates from linkage disequilibrium data

Method 5 was another short-term method of N_e estimation that utilized linkage disequilibrium (LD) data, that is, the nonrandom association of alleles at different pairs of gene loci ($\hat{r}^2$), to estimate $N_{eCLOSED}$ in the parental generation for the sample collected. Unlike Methods 1–4, Method 5 had the advantage of only requiring a single sample to estimate N_e. Method 5 can also be robust to the potential bias of selection on N_e estimation with the temporal method described above, as it is based entirely on data for the offspring sample actually being produced (Araki *et al*. 2007). Under Method 5, it is assumed that LD is the result of genetic drift within small populations that have low or potentially low N_e (Waples 1991, 2006), but migration and admixture between divergent populations can also lead to LD (Nei & Li 1973; Waples & Smouse 1990). It should also be noted that the time periods to which our LD N_e estimates apply might not be completely congruent with those of Methods 1–4, but they provide supplementary information including how N_e might have changed between 'early' or 'late' samples.

For unlinked loci, $\hat{r}^2$ is related to N_e in the equation

$$N_e = 1/[3(\hat{r}^2 - 1/S)],$$

where S is the sample size (Hill 1981; Bartley *et al*. 1992). We used Burrow's composite measure of disequilibrium (Weir 1979, 1996; Bartley *et al*. 1992) to calculate the squared correlation of allele frequencies ($\hat{r}^2$) at pairs of loci in each sample. Allelic correlations (r) were computed from samples using the LINKDOS program (Black & Krafsur 1985; Garnier-Gere & Dillmann 1992), a commonly used algorithm for estimating LD (Raymond & Rousset 1995; England *et al*. 2006; Waples 2006). The overall mean $\hat{r}^2$ for each sample was calculated as the weighted mean of values over pairs of loci [$L(L-1)/2$ pairwise comparisons] and utilized to estimate N_e. The weights for each loci pair were represented by their respective numbers of allelic comparisons. Waples (2006) has recently shown that LD N_e estimates based on the above equation can be severely biased if S is less than the true N_e, particularly if S and/or the true N_e is small (see also England *et al*. 2006). As a result, we used the bias correction outlined in Waples (2006; random mating model for $S \geq 30$ or $S \leq 30$, depending on the sample) to correct our overall mean $\hat{r}^2$ values ($\hat{r}^{2\prime}$) and resulting N_e estimates. As each of the samples collected within salmon and trout populations was from a brood year rather than from the entire generation, the LD N_e estimates were actually for the effective number of breeders (N_b) in the sampled year rather than N_e per generation. As a result, we converted N_b estimates to N_e for each population sample using the generation times outlined above as other studies have carried out (Ardren & Kapuscinski 2003). To increase the overall breadth of the general comparison of N_e estimates from Method 5 with those of other $N_{eCLOSED}$ methods, we also estimated LD N_e for three additional trout populations where temporal replicates were available (VEJ, KAR, KOV; see Table 1). Parametric 95% CI for LD N_e estimates were computed using equation 12 from Waples (2006).

Long-term effective population size (N_e) estimates

Method 6 was a long-term method of N_e estimation based on coalescent theory (Beerli & Felsenstein 2001). It assumed that populations had reached equilibrium between migration and drift and that populations had constant sizes and exchanged constant gene flow over the coalescent period ($\approx 4N_e$ generations). These assumptions are somewhat simplistic but Method 6 can provide additional information on N_e outside of the time periods of samples.

Coalescent-based N_e estimates for both sampling periods within populations (Lac Saint-Jean, circa 1980 and 1994; Bornholm, circa 1950 and 1997) were calculated separately using MIGRATE 2.1.3 (Beerli 2004a). This likelihood program jointly estimates N_e within each population as well as the effective number of migrants (N_em) into each population from all other populations, by estimating allele genealogies using MCMC sampling. Unlike traditional

F-statistics, MIGRATE also has the advantage of not assuming equal N_e and N_em among populations. When running MIGRATE on each of four data sets (Lac Saint-Jean: circa 1980, 1994; Bornholm circa 1950, 1997), the following parameters were used in a four population matrix: Brownian motion approximation to a ladder model (Ohta & Kimura 1973), 15 short chains (10 000 sampled, 1000 recorded) and 2 long chains (25 000 sampled, 5000 recorded; number of discards trees per chain: 10 000). For each data set, MIGRATE was run three times to assure that final chains were estimating the same values of Θ, a parameter related to N_e ($\Theta = 4N_e\mu$). Starting values of Θ and N_em for each run incorporated the maximum-likelihood values for these parameters in the previous run; we report results from the final run. Values of Θ were converted to N_e using mutation rates (μ) of 0.001 and 0.0005, based on common values (Estoup & Angers 1998; Ellegren 2000).

Effective metapopulation size ($\mathrm{metaN_e}$*) — Bornholm*

Estimates of N_e are biased if applied to a subpopulation with considerable genetic connection to other subpopulations within a larger metapopulation (Waples 2002a). Metapopulation N_e (*meta*N_e) depends on several factors relating to population subdivision; it can be either greater or less than the sum of subpopulation N_e comprising the metapopulation (Hedrick & Gilpin 1997; Whitlock & Barton 1997). There was little a priori evidence that Lac Saint-Jean salmon had strongly connected subpopulations (Tessier & Bernatchez 1999), but three observations from Østergaard *et al*. (2003) led us to carry out exploratory estimates of *meta*N_e for Bornholm trout: (i) recurrent extinction/recolonization events in several rivers; (ii) greater temporal differentiation within rivers than spatial differentiation between rivers, implying that the genetic dynamics of the system were best explained at the spatial scale of the entire island; (iii) small subpopulation N_e and large *m* using Method 4.

Approach 1. We pooled all samples collected circa 1950s and 1990s (Table 1), based on the much greater temporal differentiation within populations than spatial differentiation between populations. We then estimated temporal *meta*$N_{e\mathrm{CLOSED}}$ on Bornholm, using Methods 1–3 as described above. This integration of samples likely improved the precision of estimates by increasing sample sizes, even though samples were available from seven rivers in the 1990s but only four rivers in the 1950s (Table 1). Also, of the four rivers where 1950s and 1990s samples were available, three (VE, BL, TE) are larger and presumably more productive than other island rivers (Østergaard *et al*. 2003), so the greater representation of these rivers in each temporal sample might approximate proportional allelic changes occurring over time in the entire metapopulation. Note that results for Methods 1–3 were very similar using all 1990s samples and only 1990s samples from the four rivers that also had 1950s samples. Owing to the large number of comparisons involving N_e in our study, we elected not to include a similar *meta*$N_{e\mathrm{CLOSED}}$ estimate based on LD data.

Approach 2. This approach had the same pooling system of samples as Approach 1, but assumed that migration occurs from outside anadromous trout populations. We thus adopted Method 4 as described above to jointly estimate temporal *meta*$N_{e\mathrm{OPEN}}$ and *meta-m* (the generational migration rate to the metapopulation from outside sources). We included six outside trout populations as most likely sources of migrants to the island, based on knowledge of their *N*, feeding migrations and geographic proximity to Bornholm (Table 1). We employed Method 4 with samples from all source populations pooled. Note that considering each outside population as the source separately (i.e. a total of seven independent times) yielded highly congruent results.

Approach 3. We explored different scenarios under three contrasting theoretical metapopulation models (Wright 1943; Whitlock & Barton 1997; Nunney 1999), based on component N_e of different subpopulations, to estimate *meta*$N_{e\mathrm{CLOSED}}$ (details in Appendix II). In addition to assuming that Bornholm was closed to migration, these models also assumed that the true number of subpopulations comprising the metapopulation was known. Our sampling plan did not include every stream that trout inhabit, but we note that 25/42 island streams are uninhabited by the species, and most rivers with temporally consistent trout production were sampled at least once. Our scenarios thus considered three subpopulation numbers: 4, 8, and 12 (Appendix II).

Estimates of gene flow (effective number of migrants, $\mathrm{N_em}$*; migration rate,* m*)*

Gene flow and migration rate among populations was estimated using three main approaches, again since each makes different assumptions and has different properties.

First, we applied Wright's (1951) island model of population structure $F_{\mathrm{ST}} = 1/(4N_em + 1)$, to estimate the effective number of migrants (N_em) between population pairs in each data set. We then converted these values to proportions of migrants (*m*) by substituting the mean of N_e values of the two populations from N_e Methods 1–3. This substitution of short-term N_e values to obtain estimations of *m* assumed that N_e estimates had been temporally stable over the long-term. In these three cases, F_{ST} was taken as the F_{ST} between population pairs circa 1980 and 1994 (for Lac Saint-Jean) and circa 1950 and 1997 (for Bornholm).

Wright's island model has been routinely criticized for its underlying assumptions, including that populations have the same and constant sizes and exchange equal numbers of migrants and that populations have approached equilibrium between gene flow and genetic drift (Whitlock & McCauley 1999). Such conditions do not typify many natural systems (e.g. Hutchison & Templeton 1999), but geneflow estimates from Wright's model are nevertheless useful for providing a benchmark of comparison with other methods and traditional studies (Neigel 2002).

Second, we estimated long-term N_em for the same sampling periods of each data set using MIGRATE. We also converted these N_em to m estimates by summing unidirectional N_em values for each population pair generated by MIGRATE and then dividing this sum by the sum of their long-term N_e.

Third, we estimated m for the same sampling periods that specifically considered short-term gene flow (the past one to three generations), using BAYESASS 1.1 (Wilson & Rannala 2003). This Bayesian method incorporated the fact that immigrants show temporary disequilibrium in their genotypes relative to the focal population, allowing their identification as immigrants or offspring of immigrants. BAYESASS yields unidirectional estimates of m for each population pair which we firstly averaged for comparisons with other methods. Unlike the other approaches, BAYESASS does not assume migration–drift equilibrium, an assumption frequently violated in natural populations (Whitlock & McCauley 1999). Initial runs showed that convergence was reached using at least 1×10^7 MCMC iterations; for the final analysis, we used 3×10^7 iterations of which 1×10^7 were for the burn-in.

From data of MIGRATE and BAYESASS, total m into each population i for 'early' and 'late' sampling periods was calculated as

$$m_i = \sum_{j \neq i} m_{ji}$$

where m_{ji} is the migration rate from population j into i. Total m (and 95% CI) is also outputted using Method 3 above (i.e. joint estimation of N_e and m); these estimates (m') were converted using actual numbers of generations between sampling periods (as above for N_e) by

$$m = 1 - e^{(T'/T)\log(1-m')}$$

Results

Short-term estimates of N_e *with different methods*

Point estimates of short-term N_e ranged from 8 to 354 for Methods 1–4; these were virtually always larger with wider CI in salmon than trout populations (Fig. 2). Globally, N_e estimates within populations were strongly correlated

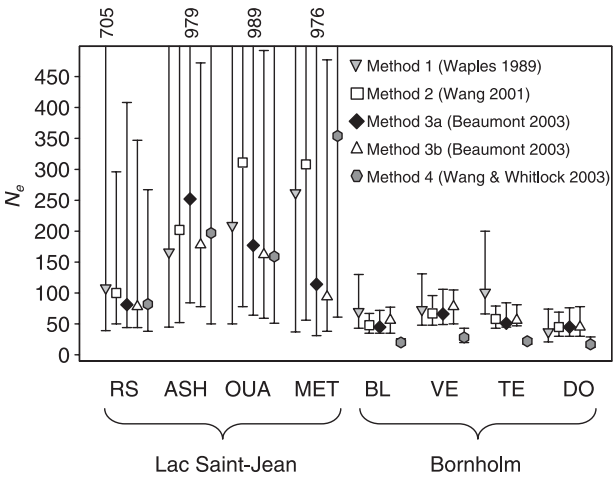

Fig. 2 Estimates of short-term effective population sizes in Lac Saint-Jean Atlantic salmon (*Salmo salar*) populations (circa 1980–1994) and Bornholm brown trout (*Salmo trutta*) populations, (circa 1950–1997) according to four different temporal methods (see Materials and Methods for explanations of each method). The 95% confidence intervals are depicted as bars. Upper CIs that exceeded 500 lead off the graph, and unless labelled at the top, were infinity. For the Bornholm data, Method 1 (Waples 1989) point estimates involve only the two most temporally separated samples. Method 3a: $N_{e\mathrm{MAX}} = 1000$; Method 3b: $N_{e\mathrm{MAX}} = 500$.

between Methods 1–4 (all Spearman's $r = 0.84–0.98$, $P < 0.05$), although less so for salmon than trout populations. For instance, no particular methods provided lower or higher N_e estimates relative to others in salmon, indicating that the major discrepancies between methods were population specific. In particular, whereas all four methods yielded N_e values of 78 to 108 in RS, N_e ranged from 94 to 354 in MET. Closer inspection of N_e estimates in MET also revealed the intriguing result that $N_{e\mathrm{OPEN}}$ (Method 4) was higher than $N_{e\mathrm{CLOSED}}$ (Methods 1–3). In contrast, $N_{e\mathrm{CLOSED}}$ estimates were very consistent within all trout populations (Fig. 2). A main inconsistency among methods across all trout populations were continuously lower $N_{e\mathrm{OPEN}}$ estimates (Method 4) (Fig. 2). Changing the Bayesian maximum N_e ($N_{e\mathrm{MAX}}$) from 1000 to 500 using Method 3 (Beaumont 2003) had no appreciable effect on point estimates of $N_{e\mathrm{CLOSED}}$ in any population except for ASH, where $N_{e\mathrm{CLOSED}}$ decreased from 252 to 178 (Fig. 2). Upper 95% CI approached $N_{e\mathrm{MAX}}$ in all cases within salmon populations, but changed little irrespective of the assumed $N_{e\mathrm{MAX}}$ within trout populations; lower CI were virtually unchanged when altering $N_{e\mathrm{MAX}}$ (Fig. 2).

In salmon, altering the allele frequencies of the source population of immigrants under Method 4 (Wang & Whitlock 2003) for RS, ASH and OUA (because of their closer genetic relationships: see Materials & Methods), had no appreciable effect on $N_{e\mathrm{OPEN}}$ estimates. Specifically,

including all three other populations in the system as sources vs. only including the two other populations in this closely related group yielded comparable N_{eOPEN} estimates [RS: 82 (38–267) vs. 80 (35–251); ASH: 197 (50–8) vs. 187 (47–8); OUA: 159 (51–1503) vs. 149 (50–1278)].

Short-term estimates of linkage disequilibrium N_e

Using Method 5, most point estimates of LD N_e ranged from 103 to 239 in Lac Saint-Jean salmon populations and from 98 to 490 in Bornholm trout populations (Table 2). Exceptions were N_e estimates of 11 for the MET 1994 sample, 4235 for the BL 1950 sample, and 1038 for the TE 1951 sample (Table 2). Excluding these three exceptions (of 21 samples), ratios between LD N_e and temporal method $N_{eCLOSED}$ estimates from Methods 1–3 were respectively, over all populations, slightly greater or less than one in salmon populations (± 1SE: 0.96 ± 0.08; 0.87 ± 0.09; 1.15 ± 0.11), but three to four times greater than one in trout populations (± 1SE: 2.97 ± 0.14; 3.84 ± 0.20; 4.08 ± 0.23) (Table 2). Note that LD N_e/temporal method $N_{eCLOSED}$ ratios in other large, mainland trout populations (VEJ, KAR, KOV) were most similar to those of Lac Saint-Jean salmon populations (± 1SE: 0.70 ± 0.06, for Method 2) (Table 2). The 95% CIs for LD N_e estimates were generally equal to or wider than temporal method estimates (Fig. 2; Table 2). LD N_e and temporal method N_e estimates were not correlated within populations (Method 5 'early' and 'late' samples vs. Methods 1–3: Spearman's $r = 0.19–0.26$, $P > 0.54$, and Spearman's $r = –0.57–0.40$, $P > 0.15$, respectively, in three of three comparisons), nor were 'early' and 'late' LD N_e estimates within populations (Spearman's $r = 0.05$, $P = 0.93$). 'Late' sample LD N_e estimates were lower than 'early' samples in three of four salmon populations, but only one of four trout populations showed a similar trend (Table 2).

Long-term estimates of N_e

Depending on the mutation rate (μ) assumed, coalescent-based long-term estimates of N_e from Method 6 were on the order of 2 to 10 times larger than short-term estimates from temporal or LD methods (Methods 1–5) in different populations, and larger in salmon than trout populations (Figs 2 and 3; Table 2). Notable exceptions were MET, in which long- and short-term N_e estimates overlapped strongly (excluding the LD N_e estimate for 1994) and Bornholm trout populations, where long-term N_e estimates overlapped with point estimates of LD N_e in several cases (Fig. 3; Table 2). Long-term N_e estimates were also very consistent between sampling years in salmon populations (circa 1980 vs. 1994), whereas they were more variable within trout populations (circa 1950 vs. 1997) (Fig. 3). As a result of these overall patterns, long- and short-term

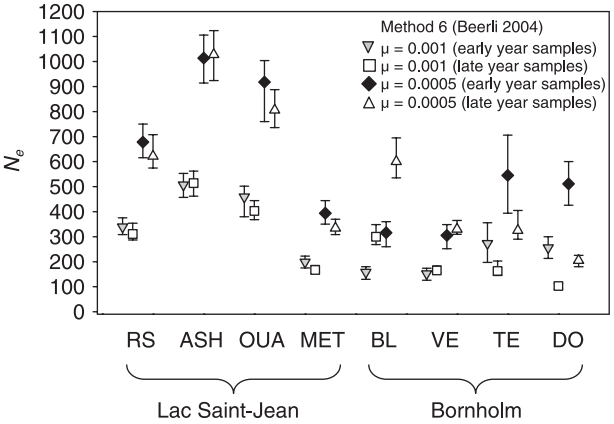

Fig. 3 Estimates of long-term effective population sizes in Lac Saint-Jean Atlantic salmon (*Salmo salar*) populations and Bornholm brown trout (*Salmo trutta*) populations, according to Beerli (2004b). The 95% confidence intervals are depicted as bars. Early year samples, circa 1980 (Lac Saint-Jean) or circa 1950 (Bornholm); late year samples, 1994 (Lac Saint-Jean) or 1997 (Bornholm). Mutation rate, μ.

N_e estimates were only sometimes correlated within populations (Method 6 'early' samples vs. Methods 1–4: Spearman's $r = 0.40–0.65$, $P < 0.05$ in 1 of 4 comparisons; Method 6 'late' samples vs. Methods 1–4: Spearman's $r = 0.595–0.77$, $P < 0.05$ in 4 of 4 comparisons; Method 6 'early' or 'late' samples vs. Method 5: Spearman's $r = 0.14–0.16$, $P < 0.05$ in 0 of 2 comparisons; based on only the earliest and latest temporal samples for Bornholm trout populations).

Effective metapopulation size ($metaN_e$*) — Bornholm*

Point estimates of $metaN_{eCLOSED}$ for the entire system of trout subpopulations on Bornholm ranged from 212 to 326 (Approach 1, Methods 1–3) (Table 3). Lower 95% CI were no smaller than 146 and upper 95% CI were no larger than 377 with these methods (Table 3). The estimate of $metaN_{eOPEN}$ (Approach 2, Method 4) was slightly lower (190) and had a smaller upper 95% CI (251), with a low estimated migration rate (m) to the island of 2.8% (Table 3). With Approach 3, estimates of $metaN_{eCLOSED}$, based on three contrasting models for estimating subpopulation N_e components, varied depending on the number of subpopulations assumed in the metapopulation. These estimates were most similar to Approaches 1–2 assuming four subpopulations under Wright's island model and Nunney's interdemic genetic drift model, or assuming 8–12 subpopulations under plausible values of m (0.05–0.15) and e (0.1–0.2) under Whitlock & Barton's extinction–recolonization model (Table 3).

Table 2 Estimates of effective numbers of breeders (N_b) and converted estimates of effective population size ($N_e = GN_b$, where G means generation time in years) using linkage disequilibrium (LD) data. Point estimates of LD N_e are compared to Methods 1–3a for estimating temporal $N_{eCLOSED}$ (Waples 1989; Wang 2001; Beaumont 2003 $N_{eMAX} = 1000$, respectively)

Sample	LD estimation N_b	G	N_e (95% CI)	Temporal estimation N_e	LD N_e: temporal N_e
S. salar: Lac Saint-Jean					
RS 1970	39.2	5.537	217 (40–∞)	108, 100, 81	2.01, 2.17, 2.68
RS 1994	19.8	5.537	109 (29–∞)	108, 100, 81	1.01, 1.09, 1.35
ASH 1978	27.8	5.440	151 (34–∞)	166, 202, 252	0.91, 0.75, 0.60
ASH 1994	18.8	5.440	103(30–2056)	166, 202, 252	0.62, 0.51, 0.41
OUA 1980	25.7	5.247	135 (31–∞)	209, 311, 177	0.65, 0.43, 0.76
OUA 1994	45.6	5.247	239 (36–∞)	209, 311, 177	1.14, 0.77, 1.35
MET 1981	17.5	6.076	106 (31–3918)	262, 308, 114	0.40, 0.34, 0.93
MET 1994	1.8	6.076	11 (9–44)	262, 308, 114	0.04, 0.04, 0.10
S. trutta: Bornholm					
VE 1950	67.2	3.5	235 (35–∞)	73, 67, 66	3.22, 3.51, 3.56
VE 1992	42.3	3.5	148 (15–∞)	73, 67, 66	2.03, 2.21, 2.24
VE 1997	77.4	3.5	271 (36–∞)	73, 67, 66	1.68, 4.04, 4.11
BL 1944	29.8	3.5	104 (24–∞)	70, 48, 45	1.49, 2.17, 2.31
BL 1950	1210.0	3.5	4235 (44–∞)	70, 48, 45	60.50, 88.23, 94.11
BL 1997	106.0	3.5	371 (39–∞)	70, 48, 45	5.30, 7.73, 8.24
DO 1966	27.9	3.5	98 (18–∞)	37, 45, 45	2.65, 2.18, 2.18
DO 1992	48.1	3.5	168 (13–∞)	37, 45, 45	4.54, 3.73, 3.73
DO 1997	43.2	3.5	151 (30–∞)	37, 45, 45	4.08, 3.36, 3.36
TE 1944	139.9	3.5	490 (34–∞)	101, 58, 51	4.85, 8.45, 9.61
TE 1951	296.7	3.5	1038 (43–∞)	101, 58, 51	10.28, 17.90, 20.35
TE 1992	30.2	3.5	106 (15–∞)	101, 58, 51	1.05, 1.83, 2.08
TE 1997	50.5	3.5	177 (32–∞)	101, 58, 51	1.75, 3.05, 3.47
GR 1997	47.0	3.5	164 (27–∞)		NA
LAE 1997	115.8	3.5	405 (40–∞)		NA
BA 1997	27.3	3.5	96 (22–∞)		NA
S. trutta: other					
VEJ 1910	47.3	3.5	166 (26–∞)	708*	0.23
VEJ 1998	111.4	3.5	390 (40–∞)	708*	0.55
KAR 1912	63.4	3.5	222 (26–∞)	665*	0.33
KAR 1951	142.7	3.5	499 (41–∞)	665*	0.75
KAR 1993	68.7	3.5	240 (34–∞)	665*	0.36
KAR 1996	166.1	3.5	581 (56–∞)	665*	0.87
KOV 1953	108.9	3.5	381 (39–∞)	445*	0.86
KOV 1996	215.8	3.5	755 (62–∞)	445*	1.70

NA, not applicable because temporal N_e estimates were not available for a comparison; *from Østergaard *et al*. (2003) and based on Wang (2001).

Temporal gene flow and migration rates

Although the magnitude of geneflow and migration-rate estimates (N_em, m) among population pairs varied with the method employed, these estimates were correlated between most methods (Fig. 4, Tables 4–6). As suspected from overall F_{ST} values obtained in previous studies, population pair N_em and m estimates were also usually several times larger between trout populations than salmon populations regardless of the method used (Fig. 4, Table 4), as were total m estimates into trout populations (Table 6). A notable exception to consistency between methods was a general lack of correlation between coalescent-based population-pair estimates of Beerli (2004a) and all other methods, particularly for N_em, and most likely with the Bornholm trout population system (Fig. 4, Table 5). Similarly, N_em and m estimates from the same method were correlated between temporal periods with the exception of Beerli (2004a) (Fig. 4, Table 5).

Of the 24 total unidirectional 'long'- and 'short'-term N_em or m estimates within population pairs in each system, 14 and 15 were asymmetric (i.e. where 95% CI did not overlap) in salmon and trout, respectively (Table 7). In salmon, these asymmetries had some consistent patterns. Even

Table 3 Exploratory metapopulation effective population size ($metaN_e$) estimates for Bornholm trout, under different approaches (see Materials & Methods and Appendix for details). Estimates of $metaN_e$ for metapopulation models of Wright and Nunney are the ranges yielded from estimating subpopulation N_e with Methods 1–3. Note that the migration model assumed refers to the metapopulation as a whole

Approach, reference, details	Data	Migration model assumed	$metaN_e$	*meta-m*	
1 Waples (1989)	Pooled data	Closed to migration	212 (146–314)		
1 Wang (2001)	Pooled data	Closed to migration	294 (229–377)		
1 Beaumont (2003), $N_{e\mathrm{MAX}}$ 500	Pooled data	Closed to migration	249 (199–341)		
1 Beaumont (2003), $N_{e\mathrm{MAX}}$ 1000	Pooled data	Closed to migration	326 (202–340)		
2 Wang & Whitlock (2003)	Pooled data	Migration; sources combined	190 (146–251)	0.028 (0.017–0.044)	
			$metaN_e$, subpopulations assumed		
			4	8	12
3 Wright (1943)	Subpopulation N_e	Closed to migration	218–281	436–562	654–843
3 Nunney (1999)	Subpopulation N_e	Closed to migration	203–262	406–524	609–786
3 Whitlock & Barton (1997), $m = 0.05$, $e = 0.1$	Subpopulation N_e	Closed to migration	85	171	256
3 Whitlock & Barton (1997), $m = 0.05$, $e = 0.2$	Subpopulation N_e	Closed to migration	68	137	205
3 Whitlock & Barton (1997), $m = 0.1$, $e = 0.1$	Subpopulation N_e	Closed to migration	114	228	341
3 Whitlock & Barton (1997), $m = 0.1$, $e = 0.2$	Subpopulation N_e	Closed to migration	57	114	171
3 Whitlock & Barton (1997), $m = 0.15$, $e = 0.1$	Subpopulation N_e	Closed to migration	68	137	205
3 Whitlock & Barton (1997), $m = 0.15$, $e = 0.2$	Subpopulation N_e	Closed to migration	49	98	146

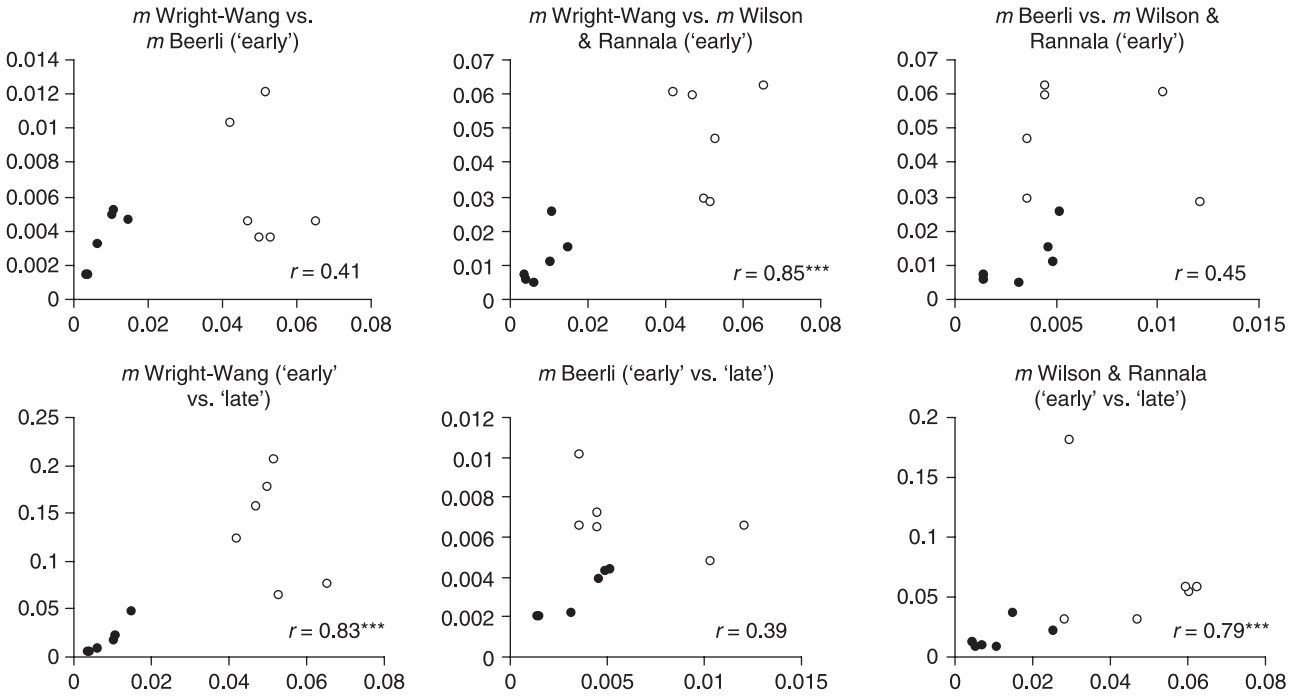

Fig. 4 Examples of relationships between migration rate (m) estimates generated from different methods. Estimates of m are those presented in Table 4. Correlation coefficients (r) for all methods and time periods are found in Table 5. Filled circles are for Lac Saint-Jean Atlantic salmon (*Salmo salar*) population pairs and open circles are for Bornholm brown trout (*Salmo trutta*) population pairs. ***$P < 0.001$. 'early', early year samples (circa 1994 Lac Saint-Jean; circa 1997 Bornholm). Note that the range of m values on x and y axes are not equivalent across different graphs.

Table 4 Generational geneflow and migration-rate estimates between each pair of *Salmo salar* and *Salmo trutta* populations. For N_em Beerli and m Beerli, only values based on a mutation rate of μ = 0.001 are presented

Population pairs	N_em Wright*†	N_em Beerli*†	m Wright–Waples*†	m Wright–Wang*†	‡m Wright–Beaumont*†	m Beerli*†	m Wilson and Rannala*†
S. salar							
RS-ASH	1.63, 3.27	4.43, 3.64	0.0119, 0.0187	0.0108, 0.0217	0.0098, 0.0154	0.0052, 0.0044	0.0255, 0.0220
RS-OUA	2.15, 3.48	3.93, 3.05	0.0136, 0.0189	0.0105, 0.0169	0.0167, 0.0233	0.0049, 0.0043	0.0110, 0.0080
RS-MET	1.30, 1.82	1.70, 1.03	0.0070, 0.0079	0.0064, 0.0089	0.0134, 0.0149	0.0032, 0.0022	0.0045, 0.0122
ASH-OUA	3.78, 12.25	4.48, 3.55	0.0202, 0.0295	0.0148, 0.0478	0.0176, 0.0255	0.0046, 0.0039	0.0152, 0.0361
ASH-MET	0.91, 1.34	1.02, 1.40	0.0043, 0.0043	0.0036, 0.0053	0.0050, 0.0050	0.0014, 0.0020	0.0071, 0.0100
OUA-MET	1.25, 1.56	0.94, 1.15	0.0053, 0.0048	0.0040, 0.0051	0.0086, 0.0077	0.0014, 0.0020	0.0055, 0.0085
S. trutta							
VE-BL	2.42, 7.11	3.21, 2.23	0.0339, 0.0993	0.0422, 0.1235	0.0437, 0.1280	0.0103, 0.0048	0.0605, 0.0545
VE-DO	3.66, 4.30	1.83, 1.93	0.0665, 0.0781	0.0653, 0.0767	0.0659, 0.0774	0.0045, 0.0072	0.0625, 0.0580
VE-TE	3.32, 3.99	1.51, 3.31	0.0382, 0.0458	0.0531, 0.0638	0.0568, 0.0682	0.0036, 0.0101	0.0470, 0.0305
BL-DO	2.18, 7.33	1.87, 2.63	0.0409, 0.1369	0.0471, 0.1575	0.0486, 0.1628	0.0045, 0.0065	0.0595, 0.0580
BL-TE	2.64, 9.36	1.54, 3.06	0.0309, 0.1095	0.0498, 0.1767	0.0550, 0.1951	0.0036, 0.0066	0.0295, 0.1810
DO-TE	2.66, 10.62	6.40, 1.75	0.0554, 0.2212	0.0516, 0.2062	0.0554, 0.2212	0.0121, 0.0066	0.0285, 0.0310

*Early year samples (Lac Saint-Jean circa 1980; Bornholm circa 1950); †late year samples (Lac Saint-Jean circa 1994; Bornholm circa 1997). ‡assuming N_{eMAX} = 1000 from Beaumont (2003).

Table 5 Spearman rank correlation coefficients (above the diagonal; statistical significance below the diagonal) between geneflow or migration-rate estimates from various methods for each population pair in Table 4

	Early vs. late years	N_em Wright*†	N_em Beerli*†	m Wright–Waples*†	m Wright–Wang*†	‡m Wright–Beaumont*†	m Beerli*†	m Wilson and Rannala*†
Early vs. late years		0.83	0.42	0.89	0.83	0.83	0.39	0.79
N_em Wright*†	***		0.44, 0.44	0.79, 0.80	0.84, 0.84	0.84, 0.85	0.45, 0.57	0.65, 0.74
N_em Beerli*†	NS	NS NS		0.40, 0.25	0.45, 0.30	0.21, 0.27	0.91, 0.40	0.19, 0.29
m Wright–Waples*†	***	*** ***	NS NS		0.94, 0.98	0.94, 0.99	0.58, 0.78	0.72, 0.78
m Wright–Wang*†	***	*** ***	NS NS	*** ***		0.98, 0.99	0.41, 0.80	0.85, 0.83
‡m Wright–Beaumont*†	***	*** ***	NS NS	*** ***	*** ***		0.35, 0.80	0.80, 0.79
m Beerli*†	NS	NS*	***NS	* ***	NS***	NS***		0.45, 0.66
m Wilson and Rannala*†	***	** **	NS NS	** ***	*** ***	*** ***	NS**	

*Early year samples (Lac Saint-Jean circa 1980; Bornholm circa 1950); †late year samples (Lac Saint-Jean circa 1994; Bornholm circa 1997). ‡assuming N_{eMAX} = 1000 from Beaumont (2003). NS, nonsignificant, *P < 0.05, **P < 0.01, ***P < 0.001.

Table 6 Total migration rates (m) into each salmon and trout population estimated from three methods (and 95% CI in parentheses for Wang & Whitlock 2003). For m Beerli, only values based on a mutation rate of μ = 0.001 are presented

Population	m Beerli*†	m Wilson and Rannala*†	m Wang and Whitlock
S. salar			
RS	0.0034, 0.0044	0.0130, 0.0590	0.0222 (0.0060–0.0691)
ASH	0.0020, 0.0021	0.0720, 0.0702	0.0080 (0.0010–0.0324)
OUA	0.0026, 0.0027	0.0400, 0.0460	0.0559 (0.0060–0.0850)
MET	0.0018, 0.0016	0.0120, 0.0160	0.0006 (0.0004–0.0035)
S. trutta			
VE	0.0042, 0.0062	0.2250, 0.1700	0.1585 (0.0975–0.2510)
BL	0.0063, 0.0036	0.1720, 0.1710	0.2924 (0.1955–0.4221)
DO	0.0053, 0.0063	0.1680, 0.1760	0.3281 (0.1645–0.6289)
TE	0.0041, 0.0060	0.0100, 0.2730	0.2694 (0.1841–0.3911)

*Early year samples (Lac Saint-Jean circa 1980; Bornholm circa 1950); †late year samples (Lac Saint-Jean circa 1994; Bornholm circa 1997).

Table 7 Unidirectional geneflow and migration-rate estimates over time within population pairs of *Salmo salar* and *Salmo trutta*. The 95% confidence intervals are in parentheses for Beerli (2004a), while Wilson & Rannala (2003) estimates contain standard deviations in parentheses. Asymmetric N_em or m estimates (where 95% CI do not overlap) are highlighted in bold font. The direction of gene flow/migration is as follows: the first population listed into the second population listed (i.e. RS-ASH, RS into ASH)

	Lac-Saint Jean data set, *S. salar*					
	RS and ASH		RS and OUA		RS and MET	
Method of asymmetric gene flow or migration	RS-ASH	ASH-RS	RS-OUA	OUA-RS	RS-MET	MET-RS
N_em Beerli*	1.44 (1.27–1.63)	**2.99 (2.70–3.40)**	2.20 (2.12–2.57)	1.73 (1.81–2.42)	0.47 (1.65–2.37)	**1.23 (1.05–1.84)**
N_em Beerli†	1.69 (1.40–1.92)	1.95 (1.73–2.19)	1.36 (1.22–1.51)	1.69 (1.48–1.79)	0.38 (0.31–0.47)	**0.65 (0.52–0.80)**
m Wilson and Rannala*	**0.046 (0.028)**	0.005 (0.006)	**0.018 (0.02)**	0.004 (0.005)	0.005 (0.007)	0.004 (0.006)
m Wilson and Rannala†	0.011 (0.013)	**0.031 (0.021)**	0.008 (0.009)	0.008 (0.009)	0.003 (0.005)	**0.020 (0.016)**
	Lac-Saint Jean data set, *S. salar* (*continued*)					
	ASH and OUA		ASH and MET		OUA and MET	
	ASH-OUA	OUA-ASH	ASH-MET	MET-ASH	OUA-MET	MET-OUA
N_em Beerli*	2.14 (1.81–2.36)	2.34 (2.13–2.73)	0.46 (1.50–2.18)	0.56 (1.83–2.73)	0.35 (1.15–1.77)	**0.59 (1.89–2.85)**
N_em Beerli†	2.10 (1.84–2.35)	1.45 (1.42–1.92)	0.35 (0.29–0.47)	**1.05 (0.90–1.22)**	0.46 (0.39–0.59)	**0.69 (0.61–0.84)**
m Wilson and Rannala*	0.014 (0.018)	0.016 (0.017)	0.004 (0.005)	**0.010 (0.011)**	0.003 (0.005)	**0.008 (0.007)**
m Wilson and Rannala†	0.025 (0.020)	**0.047 (0.025)**	0.009 (0.008)	0.011 (0.012)	0.004 (0.005)	**0.013 (0.015)**
	Bornholm data set, *S. trutta*					
	VE and BL		VE and DO		VE and TE	
	VE-BL	BL-VE	VE-DO	DO-VE	VE-TE	TE-VE
N_em Beerli*	**2.40 (1.95–2.51)**	0.81 (0.68–0.85)	**1.15 (0.98–1.21)**	0.69 (0.57–0.73)	0.46 (0.36–0.50)	**1.06 (0.88–1.11)**
N_em Beerli†	0.77 (0.58–0.87)	**1.46 (1.05–1.64)**	1.10 (0.87–1.16)	0.83 (0.71–1.04)	1.51 (1.25–1.94)	1.81 (1.46–2.27)
m Wilson and Rannala*	0.056 (0.053)	0.065 (0.061)	0.056 (0.062)	0.069 (0.070)	0.003 (0.004)	**0.091 (0.012)**
m Wilson and Rannala†	0.053 (0.0603)	0.056 (0.059)	0.060 (0.063)	0.058 (0.061)	0.005 (0.004)	**0.056 (0.055)**
	Bornholm data set, *S. trutta* (*continued*)					
	BL and DO		BL and TE		DO and TE	
	BL-DO	DO-BL	BL-TE	TE-BL	DO-TE	TE-DO
N_em Beerli*	**1.26 (1.09–1.33)**	0.61 (0.48–0.66)	0.55 (0.44–0.60)	**0.99 (0.82–1.05)**	3.40 (2.91–3.50)	3.00 (2.70–3.10)
N_em Beerli†	0.86 (0.69–1.14)	**1.77 (1.34–2.15)**	1.24 (0.89–1.36)	**1.82 (1.46–2.21)**	**1.14 (0.97–1.36)**	0.61 (0.48–0.82)
m Wilson and Rannala*	0.058 (0.053)	0.061 (0.066)	0.004 (0.003)	**0.055 (0.024)**	0.003 (0.004)	**0.054 (0.064)**
m Wilson and Rannala†	0.058 (0.058)	0.058 (0.056)	**0.264 (0.014)**	0.060 (0.064)	0.004 (0.004)	**0.058 (0.021)**

*Early year samples (Lac Saint-Jean circa 1980; Bornholm circa 1950); †late year samples (Lac Saint-Jean circa 1994; Bornholm circa 1997).

though MET exchanged less gene flow with the three other populations, nine asymmetries (across methods and temporal periods) were in the direction from MET into RS, into ASH or into OUA (Table 7). However, the five asymmetric m estimates between RS, ASH and OUA were not consistent among methods, temporal periods, or within certain population pairs (Table 7). Similarly, asymmetries in gene flow in trout were generally not consistent between methods, temporal periods, or within certain population pairs, with the possible exception being between TE and VE in which N_em and m was commonly higher from TE into VE (Table 7).

Discussion

Degree of congruence in short-term effective population size (N_e) estimates with the temporal method

The four temporal methods employed for estimating short-term N_e yielded point estimates that were strongly

correlated within populations. This was despite their differing mathematical properties and assumptions, the contrasting population genetic structure of our two study systems, and the modest numbers of loci used. However, two main inconsistencies among methods deserve mention. First, there was more variability in N_e estimates and greater uncertainty (reflected by larger CIs) in salmon than trout populations, even though realistic N_e values in salmon were only moderate (only 80 to 250). Second, in trout populations, N_{eOPEN} estimates were continuously lower than $N_{eCLOSED}$ estimates, despite very narrow CI and small N_e values of < 75 for all methods. Taken together, comparisons of N_e estimates from different methods within and between studies must be considered very cautiously until similar comparable studies are conducted using both greater sample sizes and numbers of loci. For instance, within salmon populations, all N_e estimates in RS ranged from 78–108. Since the census size of RS was only a few hundred individuals around the same time period of this study (M. Legault, unpublished data), such consistency reveals what the true N_e in this population likely is, even though upper 95% CI for N_e were considerably higher. Yet, had we based conclusions about N_e on any one method, we would have concluded that MET had anywhere from the largest N_e of the four salmon populations to one of the lowest. Such discrepancies are a good reminder that point estimates of N_e mean very little when upper CIs are large, unless multiple methods provide similar estimates and other sources of information (e.g. census sizes) can affirm them.

The larger CIs in salmon populations could reflect the inherently larger N_e within salmon than trout populations that we studied, since the CIs are a function of allelic frequency change, and the relationship between this change and N_e is nonlinear (Nei & Tajima 1981; Waples 1989). This is plausible given that (i) equal numbers of loci with similar allelic richness and similar heterozygosities were used in both population systems, (ii) temporal samples had similar sample sizes in both systems and (iii) similarly, N_e estimates in Bornholm trout populations based on any two rather than all temporal samples (making sample sizes even more comparable between systems when estimating N_e with the temporal method) still had very narrow CIs relative to Lac Saint-Jean salmon populations (data not shown). However, the temporal method also becomes less precise as the ratio S/N_e gets smaller (where S = sample size). Even with equal sampling effort then, a reduced S/N_e ratio, which would translate into larger CIs, would occur most often where populations have larger than smaller N_e (Waples 1989). In addition, precision for the temporal method decreases when the number of generations between samples (T) is smaller (Waples 1989), as was the case for Lac Saint-Jean salmon population samples relative to Bornholm trout samples.

With respect to trout, $N_{eCLOSED}$ estimates were very similar and follow the literature trend for uniformity when N_e is small (i.e. < 75–100: see also Miller & Waits 2003; Aspi *et al*. 2006). However, $N_{eCLOSED}$ estimates were 2.4 to 4.6 times larger than N_{eOPEN} estimates of Wang & Whitlock (2003) in all populations. A cursory ISI Web of Science literature search yielded eight other studies where N_{eOPEN} of Wang & Whitlock (2003) and at least one temporal $N_{eCLOSED}$ method were employed (Ford *et al*. 2004; Hoffman *et al*. 2004; Johnson *et al*. 2004; Consuegra *et al*. 2005; Jensen *et al*. 2005; Saillant & Gold 2006; Fraser *et al*. 2007; Watts *et al*. 2007). In all cases, $N_{eCLOSED}$ estimates were higher than N_{eOPEN}, on the order of 1.4 to in excess of 87 times. In one system, such $N_{eCLOSED}/N_{eOPEN}$ ratios were consistent in both large N_e, temporal stable populations and in smaller N_e populations with unstable genetic compositions (Fraser *et al*. 2007). To date, it appears that the only case where $N_{eCLOSED}$ and N_{eOPEN} estimates were congruent (or that $N_{eOPEN} > N_{eCLOSED}$) is within our salmon populations.

The discrepancies between N_{eOPEN} and $N_{eCLOSED}$ estimates across multiple systems and species (and different numbers of generations elapsed between temporal samples) raise questions about what methods actually yield estimates closer to 'true' N_e values. Such discrepancies could result from a variety of violations of the assumptions of different methods, including: (i) inadequate sample sizes or numbers of loci utilized; (ii) insufficient knowledge of source populations contributing migrants; (iii) inadequate knowledge of the extent of gene flow; (iv) population size fluctuations over the period that N_e was estimated; (v) the application of discrete generations to species with overlapping generations to generate N_e values; or (vi) too few generations separating temporal samples (Jorde & Ryman 1995; Waples 2002a, b; Wang & Whitlock 2003; Waples & Yokota 2007). Lower N_{eOPEN} in trout might have also arisen because Wang & Whitlock's (2003) model assumes that constant immigration occurs from an infinite-sized source population of fixed allele frequency, but our temporal gene flow analyses indicate that this model is not even roughly applicable in Bornholm (discussed below).

Another explanation for continually lower N_{eOPEN} and $N_{eCLOSED}$ estimates in different systems relates to the fact that allelic frequency change within populations from migration depends on the extent of genetic differentiation from the source populations of migrants. Wang & Whitlock (2003) have argued that in the short term, migration results in very rapid changes in allele frequencies, and this leads to nonequilibrium conditions between migration and drift. Populations then behave as if drift changes allele frequencies quickly, so N_e is underestimated if migration is ignored. However, we suggest that this might only be true if source populations of migrants are genetically dissimilar from the receiving population. If genetic differentiation is

conversely low, migration might offset the effect of drift in the short-term, leading to an overestimation of N_e if it is ignored. Wang &Whitlock (2003) further stipulated that in the long term, assuming that migration is constant, migration and drift approach equilibrium and the pace at which allelic frequency changes occurs in a population then reflects that of the larger, *meta*N_e, leading again to an overestimate of population N_e if migration is ignored (Wang & Whitlock 2003; see also Waples 2002a). The higher $N_{e\text{CLOSED}}$ estimates in many studies would therefore imply that migration into populations is often high enough (and genetic differentiation is low enough) to lead to an overestimation of N_e with such methods. In contrast, congruent $N_{e\text{OPEN}}$ and $N_{e\text{CLOSED}}$ estimates in Lac Saint-Jean salmon populations would suggest that migration is too low in the system to have an effect on N_e estimation. On the other hand, there are clearly cases where $N_{e\text{OPEN}}$ estimates were biased downwards and with little biological sense (Hoffman *et al*. 2004). Additionally, spatial population genetic differentiation has been low or moderate in many of the above studies (F_{ST} = 0.01–0.05). This likely also renders the precise estimation of Wang and Whitlock's m challenging because nonequilibrium conditions appear to commonly confound interpretations of genetic differentiation in many systems (Hutchison & Templeton 1999; Whitlock & McCauley 1999). It is thus possible that $N_{e\text{CLOSED}}$ is generally biased upwards (in the absence of variable selection at gene loci), Wang and Whitlock's m is usually biased upwards, and/or $N_{e\text{OPEN}}$ is usually biased downwards.

No clean-cut answers currently solve these ambiguities, but their consideration merits attention in individual studies since some degree of migration between populations is likely in many systems. It is clear that the migration model of Wang and Whitlock's method is simplistic and, like Wright's island model of migration, it will likely not apply to many real world situations. Until further theoretical developments consider more complex scenarios of the role of migration on N_e estimation, we suggest that where *any* gene flow is suspected, researchers should calculate $N_{e\text{OPEN}}$ and $N_{e\text{CLOSED}}$ from at least one method, and consider interpretations of these values very cautiously. One useful approach is to consider supplementary information on the system which may facilitate conclusions regarding which methods yield more plausible estimates. For instance, in two Atlantic salmon populations, Fraser *et al*. (2007) argued that $N_{e\text{OPEN}}$ estimates were more biologically realistic than much higher $N_{e\text{CLOSED}}$ ones because each population showed continual signs of bottlenecking and linkage disequilibrium (a common phenomenon in small N_e populations) but no loss of allelic richness for up to six generations. Alternatively, one could integrate information over multiple samples collected within populations or over multiple genetic markers (Waples 2002a). Still another approach is to consider estimates of *meta*N_e where subpopulations are suspected to be closely linked temporally and spatially to one another (see below).

Degree of congruence between short-term N_e *based on temporal versus linkage disequilibrium data*

Full congruence between N_e estimates from the temporal method and LD data was not expected as the time periods to which either applied were close but not identical. Encouragingly, however, point estimates of LD $N_{e\text{CLOSED}}$ were of the same order of magnitude as temporal $N_{e\text{CLOSED}}$ in both population systems (for the vast majority of samples), despite low precision in many cases (upper 95% CI of ∞). Still, $N_{e\text{CLOSED}}$ estimates from LD and temporal methods were not correlated within populations, and a salient feature of our results was the differing LD $N_{e\text{CLOSED}}$: temporal $N_{e\text{CLOSED}}$ ratios between salmon and trout populations. These ratios were generally slightly less than or greater than one in temporally stable salmon populations and other Danish trout populations known for their larger population sizes, temporal stability of genetic structure, and low gene flow (Hansen *et al*. 2002). In contrast, LD $N_{e\text{CLOSED}}$ estimates were consistently three to four times higher in temporally unstable trout populations from Bornholm, even though all trout populations in our study were genotyped at the same loci and had similar sample sizes as Bornholm trout (and similar allelic richness).

Such patterns suggest that an assumption of either the LD or temporal method for estimating N_e was violated in Bornholm. For the LD N_e method, an obvious violation again would be the assumption of no gene flow. Gene flow and resulting admixture between genetically differentiated populations will inflate LD in a sample, which will, in turn, lead to a downward bias in N_e estimation (Nei & Li 1973; Waples 2006). However, the extent to which this occurs depends on how strongly differentiated the populations are, with more differentiated population admixture leading to a greater downward bias in N_e estimates (Waples & Smouse 1990). Although this could explain cases of lower LD N_e than other temporal N_e estimates in Lac Saint-Jean salmon populations, the often much higher LD N_e estimates in Bornholm trout populations could imply that (i) strong LD is not generated from the extensive gene flow between these populations because they are strongly connected spatiotemporally; (ii) within-river N_e estimates more typify *meta*N_e, again because populations are strongly connected spatiotemporally (see below); and/or (iii) violation of the assumption of no gene flow is more severe for the LD method for estimating $N_{e\text{CLOSED}}$ than for temporal methods. Notably, it is difficult to explain the higher LD N_e estimates in Bornholm trout populations based on sampling bias, because in general, the LD method

is only slightly biased upwards when sample sizes *exceed* true N_e (England *et al*. 2006; Waples 2006). Furthermore, the LD N_e method considered in this study was developed for diallelic loci and its performance has not been assessed with highly polymorphic genetic markers such as microsatellites (Waples 2006). With greater than two alleles per locus, it could be that allelic correlations become weaker and N_e estimates thereby higher. Still, in such a case, upward biases ought to have been similar for all the trout and salmon populations, again given their comparable levels of allelic richness and heterozygosity.

An alternative to an upward bias in the LD $N_{e\mathrm{CLOSED}}$ estimates within Bornholm trout populations could be instead that a violation of the assumption of no selection occurred in the system that led to a downward bias in $N_{e\mathrm{CLOSED}}$ estimates based on the temporal method. Indeed, unlike the latter, the LD method can be robust to the potential bias of selection on N_e estimation (Araki *et al*. 2007). Our data set was not conducive to rigorously assess to what degree selection could have affected the estimation of N_e with the loci employed, and we highly encourage parallel studies to do so in the future. Nevertheless, based on the ecology of Bornholm brown trout, namely the recurrent extinction/recolonization in many rivers, the patterns of unstable population structuring, the small population sizes found with individual rivers and the strong evidence for an overriding effect of gene flow on influencing population structure (Larsen 1970; Østergaard *et al*. 2003), we tentatively favour that an upward bias with the LD method is the more likely of the two possibilities.

These points reiterate that when gene flow or selection is suspected, such information needs to be considered when interpreting short-term N_e estimates from LD and temporal methods. In addition, while the LD method is potentially very useful because it requires only one sample to estimate N_e, curious biases in point estimates of LD N_e were evident occasionally (e.g. MET 1994, BL 1950). A cautionary approach to using this method would thus be to replicate sampling (e.g. years) to evaluate the congruence of LD N_e estimates from more than one sample. Temporal replication of the LD method also has an advantage of evaluating potential changes in N_e over time for genetic monitoring, although intergenerational changes in N_e can also be evaluated with Beaumont's (2003) temporal method and this has been considered elsewhere (Hansen *et al*. 2006b). For example, three of four N_e estimates from our 'late' (1994) samples of Lac Saint-Jean salmon populations were considerably lower than our early samples. These changes coincide with artificial enhancement that occurred in these populations in the late 1980s and early 1990s, in which juveniles from nonlocal populations were stocked into other rivers to supplement them (Tessier *et al*. 1997; Tessier & Bernatchez 1999).

Long-term versus short-term effective population size (N_e) estimates

Coalescent-based long-term estimates of N_e were on the order of two to ten times higher than short-term temporal N_e in the majority of studied populations. Furthermore, long- and short-term N_e estimates within populations were only correlated for certain methods. Studies have interpreted larger long-term N_e to indicate that populations have experienced recent declines (Alo & Turner 2005). This is possible in both of our study systems since populations have experienced declines over the past few decades because of human activities (e.g. fishing). However, such disparities in N_e could also be due to violations associated with the estimation of long-term N_e with MIGRATE. For instance, the program assumes discrete generations (whereas salmonids have overlapping generations), and assumes that populations have had constant sizes and constant gene flow exchange over the coalescent period ($\approx 4N_e$ generations). Additionally, although we applied mutation rates (μ) that characterize microsatellite loci in many vertebrates (Estoup & Angers 1998), there is clearly some inherent uncertainty with the estimation of μ. Interestingly, long-term N_e were closer to short-term estimates when $\mu = 1 \times 10^{-3}$, suggesting that this mutation rate perhaps applies better to fish than 5×10^{-4} (Estoup & Angers 1998). Finally, long-term N_e estimates from early versus late temporal samples were also highly congruent in salmon, but not in trout. This reiterates the importance of including temporal sampling replicates even though these are not required to obtain long-term N_e estimates.

Assumptions regarding source populations of migrants when estimating N_e

Migration likely influenced N_e estimation in at least the Bornholm trout populations, so we now consider the chief assumption underlying the current estimation of N_e and m concurrently-namely, that populations receive constant migration from an infinite source of fixed allele frequency (Wang & Whitlock 2003). For Lac Saint-Jean, RS, ASH and OUA could be considered principal sources of migrants for one another because of their closer genetic relationships (Tessier & Bernatchez 1999). Yet, excluding MET as a source population of migrants for RS, ASH and OUA had no appreciable effect on $N_{e\mathrm{OPEN}}$ estimates. This could be explained in two ways. First, while temporal changes in migrant contributions occurred via shifting asymmetries in N_em or m between RS, ASH and OUA, asymmetric N_em or m never exceeded one to two effective migrants or a few percent, respectively. Second, allele frequencies at different loci were strongly correlated within all populations over time (Tessier & Bernatchez 1999), so the genetic 'signal' of their migrants was likely stable over time. Johnson *et al*.

(2004) and Hansen *et al*. (2007) also found no appreciable effect of source population on N_e estimation with the Wang and Whitlock method when including biologically-relevant information of the likely source populations of migrants. For Bornholm, however, the assumption of constant migration from an infinite source population is untenable. Considerable changes in allele frequencies in all trout populations occurred over time (Østergaard *et al*. 2003), as did the direction of asymmetries in N_em or m that in some cases were quite large (e.g. $m > 0.05$). As previously mentioned, this could account for the discrepancies between N_{eOPEN} and $N_{eCLOSED}$ estimates. On the other hand, it is debatable whether N_e in each river from any of Methods 1–5 best describes N_e in this system, because the entire system may behave as a metapopulation (see below). In short, other studies using the Wang and Whitlock method should provide (i) ample justification for the application of its migration model in a given system, and (ii) results from modifying source populations based on biologically-relevant information, especially if some sources are unknown.

Effective metapopulation size — Bornholm

Ambiguities surrounding the relative influence of migration on N_e estimation raise issues about the scale at which N_e estimates should be generated in a given system. Several observations suggested that trout inhabiting individual rivers on Bornholm did not constitute discrete populations (see Materials & Methods). Instead, the species potentially behaved as one main unit on the entire island, with the larger, metapopulation scale being more relevant for estimating N_e and for explaining the maintenance of genetic variability within different rivers.

Our exploratory analysis of whole-island $metaN_e$ yielded several interesting results. First, regardless of whether the island was assumed to be closed to outside migration or not, $metaN_e$ estimates ranged similarly from 190 to 326 (CI 146–377), although $metaN_{eOPEN}$ was lowest. Second, outside migration (m) to the island was estimated to be low. Values of $metaN_{eOPEN}$ and m also changed little when considering each outside trout population individually as the source of migrants (data not shown). These results clearly indicate that Bornholm is essentially closed off from outside immigration, and that consistent with metapopulation structure, the temporal instability of genetic structure and high m found within individual rivers reflects genetic processes occurring on the island itself (i.e. m comes chiefly from other island rivers). In other words, outside trout populations do not appear to act as mainland sources to the island, and the $metaN_e$ represents the natural productivity of the available habitat on Bornholm, implying further that the island is not a sink in the classic sense (Dias 1996; Hanski & Gaggiotti 2004). Third, in accounting for plausible levels of extinction and recolonization on Bornholm, the most realistic $metaN_e$ model based on subpopulation N_e components (Whitlock & Barton 1997) generated similar $metaN_e$ estimates (146–341) when 12 subpopulations were assumed. This subpopulation number is realistic given that only $\approx 1/3$ of habitat patches (rivers) was sampled. Finally, even under contrasting approaches and varying the number of possible subpopulations on Bornholm considerably, most $metaN_e$ estimates did not exceed several hundred. Again, given the low m to Bornholm, the temporal changes in population genetic structure on the island are consistent with $metaN_e$ estimates. For conservation actions, these results reiterate that conserving habitats in as many rivers on Bornholm as possible, whether presently occupied or unoccupied, will be important for the persistence of trout on the island (Østergaard *et al*. 2003). More generally, the fuller integration of N_e and m estimates at different spatiotemporal scales in this system emphasizes their benefits for (i) teasing apart processes implicated in population structuring, and (ii) more rigorously validating the performance of different N_e approaches, particularly with respect to the influence of population subdivision on N_e estimation.

Degree of congruence between N_em *or* m *estimates from different methods*

Pairwise N_em or m estimates were generally several times larger between population pairs in trout than in salmon. Population pair N_em or m estimates between the two sampling periods were correlated in many cases, at least in relative terms, as in some cases their magnitude varied considerably (e.g. m_{Beerli} versus $m_{Wilson\ \&\ Rannala}$ in trout). A notable exception to the trend for correlated N_em or m estimates was between those of Beerli (2004a) and most other methods.

Like varying approaches to estimating N_e, there is currently no general consensus regarding what methods estimate gene flow best (Whitlock & McCauley 1999; Neigel 2002; Abdo *et al*. 2004). Each method we employed required multiple assumptions, some of which were likely violated within our study systems. We might interpret the long-term likelihood estimates to be more precise than traditional methods (Wright) since the latter make more unrealistic assumptions, for instance, that populations have equal N_e and exchange equal N_em (Beerli & Felsenstein 2001). Still, a recent simulation study of mitochondrial DNA sequence data found that Beerli's method often yielded inaccurate estimates of migration (Abdo *et al*. 2004), but the generality of these findings to microsatellite data in our study are unknown. We might then further interpret short-term estimates of m based on Wilson & Rannala (2003) to better depict current patterns and levels of migration among populations, since the traditional and

coalescent-based geneflow models assume migration–drift equilibrium and constant m whereas the former does not, and such equilibrium might not have been reached in either system.

Other studies have also noted discrepancies between gene-flow methods, whereas others have found no inconsistencies. For instance, both Fraser *et al*. (2004) and Hendry & Taylor (2004) also found that traditional (Wright) and maximum-likelihood (Beerli) estimates were uncorrelated. In contrast, Hänfling & Weetman (2006) found good congruence between Beerli's method and a short-term method analogous to the Wilson and Rannala method in our study (Corander *et al*. 2004). Obviously, the lack of general consensus in N_em or m estimates over different studies indicates that population genetic structure within many species deviates frequently from current theoretical geneflow models. It is thus a good reminder to compare long- and short-term gene flow with traditional and newer approaches in any system. This is particularly relevant because in many systems, human influences, recent environmental disturbances, or the recent origin of populations can have confounding effects on geneflow estimates (Hutchison & Templeton 1999; Hänfling & Weetman 2006). If anything, a comparison of different estimators can shed light on the plausible range of gene flow between populations. This will most certainly be useful in several contexts, including improving our ability to determine the consequences of a known increase or decrease of gene flow in speciation genetics (Coyne & Orr 2004) and conservation initiatives relating to translocations or supplementation (Storfer 1999).

Temporal consistencies and inconsistencies of asymmetric gene flow

If interpopulation gene flow is asymmetric, this can affect the maintenance of genetic variability among populations and provide insight into metapopulation structure (Fraser *et al*. 2004; Hindar *et al*. 2004; Manier & Arnold 2005; Brown *et al*. 2006; Hänfling & Weetman 2006; Veliz *et al*. 2006; see also Vuilleumier & Possingham 2006). Asymmetric gene flow is also important for understanding the degree to which particular populations contribute to the migrant pool and the extent to which m constrains adaptive divergence, especially in populations that are net-receivers of migrants (Dias *et al*. 1996; Fraser & Bernatchez 2005; Fraser *et al*. 2007; Hansen *et al*. 2007).

Our long-term data sets allowed us to examine the degree to which gene flow was asymmetric over time in two contrasting population systems. We found evidence for asymmetries in N_em or m between populations in both systems, and these were detectable using both long- and short-term gene-flow methods. However, these asymmetries were only sometimes temporally stable (e.g. salmon: into RS, ASH and OUA from MET). What do these results signify beyond interpretations of traditionally applied population-pair geneflow estimates? For starters, they suggest that in many cases, gene flow between natural populations is intermittent and variable. Consequently, for Bornholm trout, as an example, the lack of continual source populations of migrants reiterates the importance of conserving habitats in as many rivers on the island as possible, since none stand out that could be justifiably prioritized over others. In salmon, the temporally stable gene flow asymmetries into RS, ASH, OUA from MET are interesting because RS, ASH and OUA are differentiated from MET in putatively adaptive migratory behaviour (Potvin & Bernatchez 2001). Since such differentiation persists over time (Potvin & Bernatchez 2001) despite continual migration from MET, levels of asymmetric gene flow from MET appear insufficient to constrain adaptive divergence in RS, ASH or OUA. Thus, analyses of asymmetric gene flow also provide supplementary, indirect evidence of the role of diversifying selection in population differentiation.

Most studies considering asymmetric gene flow to date have not been temporally replicated. The temporal inconsistencies in asymmetric gene flow in our study suggest that great care should be given in their interpretation when sampling is nonreplicated. This might be less important in situations where it is biologically reasonable that asymmetries occur in the same direction because of directional dispersal vectors (e.g. river or ocean currents), but even here temporal replication would shed light on the degree of variation in the asymmetry. The inconsistent direction of asymmetries in some cases in our study could also reflect sampling variance, although most spatial and temporal samples fulfilled minimum sampling requirements for the methods we applied.

Summary and recommendations

Our empirical comparisons of different N_e and temporal geneflow methods have several implications for their integration in fundamental and applied research, and they raise some caveats:

1. Different temporal methods for estimating N_e in closed populations yield highly congruent estimates when N_e is small. For genetic monitoring (Schwartz *et al*. 2007), the outlook for estimating N_e in endangered species or in closed populations is therefore good even with modest numbers of loci and sample sizes.
2. As expected, CIs for N_e become wider as N_e values become larger and, importantly, point estimates of N_e generated by different temporal methods vary considerably for the same populations, whereas CIs are more congruent. This stresses the need for considering CIs rather than point estimates for even moderate N_e (< 250).

3 Estimating N_e is obviously more difficult in larger populations. The question is which approach to take. One could (a) include more temporal samples, (b) analyze more loci, (c) increase sample sizes or analyze more variable loci, and/or (d) analyze loci using different markers and integrate their information. Berthier *et al*. (2002) discuss how (b) or (c) might overcome some of these challenges, and Waples (2002a) discusses the usefulness of integrating information from multiple temporal samples.

4 When gene flow cannot be ruled out, short-term N_e should be estimated assuming both closed and open systems. The only current method for doing so is that of Wang & Whitlock (2003). The problem is that their migration model is too simple, so the onus will be on the researcher to provide justification for its application and to consider supplementary, biological information that may facilitate conclusions regarding which methods yield more plausible N_e estimates. Methods that simultaneously estimate N_e and gene flow for several populations with temporal samples are needed.

5 Congruence between $N_{e\text{OPEN}}$ and $N_{e\text{CLOSED}}$ estimates from temporal and linkage disequilibrium methods likely reveals that the effect of gene flow on N_e estimation is small (e.g. Lac Saint-Jean salmon populations; migration to trout populations on the island of Bornholm from outside sources). The same tenet is perhaps true for the effect of selection on N_e estimation, but this awaits further investigation.

6 Incongruence between N_e estimates from different methods must be interpreted cautiously (e.g. trout), since depending on the assumption being violated in a given population system (migration, selection), upward or downward biases can occur with either temporal or linkage disequilibrium methods.

7 More emphasis on *meta*N_e is needed in the empirical literature. As pointed out in trout, traditional estimators of N_e (e.g. the temporal method, linkage disequilibrium) become of limited use when population systems are highly dynamic. Just as there is now wide acceptance that population genetic structure should not be assumed a priori (e.g. Pritchard *et al*. 2000), it should be considered that samples collected for N_e analyses are reflective of entire populations rather than subdivided populations or metapopulations.

8 Incongruence of geneflow estimates over different studies is a good reminder to consider traditional and newer approaches when interpreting geneflow patterns in any system, and how gene flow varies over time.

9 Likewise, asymmetries in gene flow should be treated cautiously and ideally be confirmed over time before making appropriate conclusions regarding their consequences for population structure. Our study therefore reiterates the importance of temporal replication in population genetics (e.g. Waples 1998).

10 All N_e and m methods are potentially useful, but because of their varying assumptions, they may be biased even within different population systems of the same species or closely related species. Their continual integration is essential given that human influences are increasingly resulting in nonequilibrium conditions within many systems (Hedrick & Gilpin 1997; Waples 2002a).

Acknowledgements

We thank Anna M. Calvert, Eric B. Taylor, one anonymous reviewer and especially Robin S. Waples for constructive comments on a previous version of this paper. We also thank Niels G. Fritzner for providing molecular data for the MOS population. Funding for this work was provided by a Natural Sciences and Engineering Research Council of Canada (NSERC) Postdoctoral fellowship to D.J.F., a NSERC Discovery grant to L.B., and a Danish Natural Science Research Council grant to M.M.H.

References

Abdo ZAID, Crandall KA, Joyce P (2004) Evaluating the performance of likelihood methods for detecting population structure and migration. *Molecular Ecology*, **13**, 837–851.

Adkison M (1995) Population differentiation in Pacific salmon: local adaptation, genetic drift, or the environment? *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 2762–2777.

Alo D, Turner TF (2005) Effects of habitat fragmentation on effective population size in the endangered Rio Grande silvery minnow. *Conservation Biology*, **19**, 1138–1148.

Araki H, Ardren WR, Blouin MS (2007) A potential bias in the temporal method for estimating N_e in admixed populations under natural selection. *Molecular Ecology*, **16**, 2261–2271.

Ardren WR, Kapuscinski AR (2003) Demographic and genetic estimates of effective population size (N_e) reveals genetic compensation in steelhead trout. *Molecular Ecology*, **12**, 35–49.

Aspi J, Roinenen E, Ruokonen M, Kojola I, Vila C (2006) Genetic diversity, population structure, effective population size and demographic history of the Finnish wolf population. *Molecular Ecology*, **15**, 1561–1576.

Bartley D, Bagley M, Gall G, Bentley M (1992) Use of linkage disequilibrium data to estimate effective population size in hatchery and natural fish populations. *Conservation Biology*, **6**, 365–375.

Beaumont MA (2003) Estimation of population growth or decline in genetically monitored populations. *Genetics*, **164**, 1139–1160.

Beerli P (2004a) MIGRATE version 1.7.3. University of Washington, Seattle, Washington.

Beerli P (2004b) Effect of unsampled populations on the estimation of population sizes and migration rates between sampled populations. *Molecular Ecology*, **13**, 827–836.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective populations sizes in n subspopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences, USA*, **98**, 4563–4568.

Berthier P, Beaumont MA, Cornuet JM, Luikart G (2002) Likelihood-based estimation of the effective population size using temporal changes in allele frequencies: a genealogical approach. *Genetics*, **160**, 741–751.

Black CW, Krafsur ES (1985) A FORTRAN program for the calculation and analysis of two-locus linkage disequilibrium coefficients. *Theoretical and Population Genetics*, **70**, 491–496.

Brown RP, Hoskisson PA, Welton JH, Baez M (2006) Geological history and within-island diversity: a debris avalanche and the Tenerife lizard *Gallotia galloti*. *Molecular Ecology*, **15**, 3631–3640.

Campbell D, Bernatchez L (2004) Genomic scan using AFLP markers as a means to detect directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution*, **21**, 945–956.

Consuegra S, Verspoor E, Knox D, García de Leániz C (2005) Asymmetric gene flow and the evolutionary maintenance of genetic diversity in small, peripheral Atlantic salmon populations. *Conservation Genetics*, **6**, 823–842.

Corander J, Waldmann P, Marttinen P, Sillanpaa MJ (2004) BAPS 2: enhanced possibilities for the analysis of genetic population structure. *Bioinformatics*, 20, 2363–2369.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer & Associates, Sunderland, Massachusetts.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology & Evolution*, **15**, 290–295.

Dannewitz J, Maes GA, Johanneson L, Wickstrom H, Volkaert FAM, Jarvi T (2005) Panmixia in the European eel: a matter of time. *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **272**, 1129–1137.

Dias PC (1996) Sources and sinks in population biology. *Trends in Ecology & Evolution*, **11**, 326–330.

Dias PC, Verheyen GR, Raymond M (1996) Source-sink populations in Mediterranean blue tits: evidence using single-locus minisatellite probes. *Journal of Evolutionary Biology*, 9, 965–978.

Ellegren H (2000) Heterogeneous mutation processes in human microsatellite DNA sequences. *Nature Genetics*, **29**, 1–8.

England PR, Cornuet JM, Berthier P, Tallmon DA, Luikart G (2006) Estimating effective population size from linkage disequilibrium: severe bias with small samples. *Conservation Genetics*, **7**, 303–308.

Estoup A, Angers B (1998) Microsatellites and minisatellites for molecular ecology: theoretical and practical applications. In: *Advances in Molecular Ecology* (eds Muyzer G, Carvalho GR). IOS Press, Amsterdam.

Ford MJ, Teel DJ, Van Doornik DM *et al*. (2004) Genetic population structure of central Oregon Coast coho salmon (*Oncorhynchus kisutch*). *Conservation Genetics*, **5**, 797–812.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge, UK.

Faubet P, Waples RS, Gaggiotti OE (2007) Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Molecular Ecology*, **16**, 1149–1166.

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Molecular Ecology*, **10**, 2741–2752.

Fraser DJ, Bernatchez L (2005) Adaptive migratory divergence among sympatric brook charr populations. *Evolution*, **59**, 611–624.

Fraser DJ, Lippé C, Bernatchez L (2004) Consequences of unequal population size, asymmetric gene flow and sex-biased dispersal for population structure in brook charr (*Salvelinus fontinalis*). *Molecular Ecology*, **13**, 67–80.

Fraser DJ, Jones MW, McParland TL, Hutchings JA (2007) Loss of historical immigration and the unsuccessful rehabilitation of extirpated salmon populations. *Conservation Genetics*, **8**, 527–546.

Fritzner NG, Hansen MM, Madsen SS, Kristiansen K (2001) Use of microsatellite markers for identification of indigenous brown trout in a geographical region heavily influenced by stocked domesticated trout. *Journal of Fish Biology*, **58**, 1197–1210.

Garnier-Gere P, Dillmann C (1992) A computer program for testing pairwise linkage disequilibria in subdivided populations. *Journal of Heredity*, **83**, 239.

Guinand B, Lemaire C, Bonhomme F (2004) How to detect polymorphisms undergoing selection in marine fishes? A review of methods and case studies, including flatfishes. *Journal of Sea Research*, **51**, 167–182.

Hänfling B, Weetman D (2006) Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the River Sculpin, *Cottus gobio*. *Genetics*, **173**, 1487–1501.

Hansen MM, Ruzzante DE, Nielsen EE, Bekkevold D, Mensberg KLD (2002) Long-term effective population sizes, temporal stability of genetic composition and potential for local adaptation in anadromous brown trout (*Salmo trutta*) populations. *Molecular Ecology*, **11**, 2523–2535.

Hansen MM, Bekkevold D, Jensen LF, Mensberg KLD, Nielsen EE (2006a) Genetic restoration of a stocked brown trout *Salmo trutta* population using microsatellite DNA analysis of historical and contemporary samples. *Journal of Applied Ecology*, **43**, 669–679.

Hansen MM, Nielsen EE, Mensberg KLD (2006b) Underwater but not out of sight: genetic monitoring of effective population size in the endangered North Sea houting (*Coregonus oxyrhynchus*). *Canadian Journal of Fisheries and Aquatic Sciences*, **63**, 780–787.

Hansen MM, Skaala O, Jensen JF, Bekkevold D, Mensberg KLD (2007) Gene flow, effective population size and selection at major histocompatibility genes: brown trout in the Hardanger Fjord, Norway. *Molecular Ecology*, **16**, 1413–1425.

Hanski I, Gaggiotti O (2004) *Ecology, Evolution and Genetics of Metapopulations*. Academic Press, San Diego, California.

Hedrick PW, Gilpin ME (1997) Genetic effective size of a metapopulation. In: *Metapopulation Biology: Ecology, Genetics and Evolution* (eds Hanski I, Gilpin ME), pp. 165–181. Academic Press, San Diego, California.

Hendry AP, Taylor EB (2004) How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. *Evolution*, **58**, 2319–2331.

Hendry AP, Day T, Taylor EB (2001) Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. *Evolution*, **55**, 459–466.

Hill WG (1981) Estimation of effective population size from data on linkage disequilibrium. *Genetical Research*, **38**, 209–216.

Hindar K, Tufto J, Saettem LM, Balstad T (2004). Conservation of genetic variation in harvested salmon populations. *ICES Journal of Marine Science*, **61**, 1389–1397.

Hoffman EA, Scheuler FW, Blouin MS (2004) Effective population sizes and temporal stability of genetic structure in *Rana pipiens*, the northern leopard frog. *Evolution*, **58**, 2536–2545.

Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. *Evolution*, **53**, 1898–1914.

Jehle R, Wilson GA, Arntzen JW (2005) Contemporary gene flow and the spatio-temporal genetic structure of subdivided newt

populations (*Triturus cristatus*, *T-marmoratus*). *Journal of Evolutionary Biology*, **18**, 619–628.

Jensen LF, Hansen MM, Carlsson J, Loeschcke V, Mensberg KLD (2005) Spatial and temporal genetic differentiation and effective population size of brown trout (*Salmo trutta*) in small Danish rivers. *Conservation Genetics*, **6**, 615–621.

Johnson JA, Bellinger MR, Toepfer JE, Dunn P (2004) Temporal changes in allele frequencies and low effective population size in greater prairie-chickens. *Molecular Ecology*, **13**, 2617–2630.

Jorde P, Ryman N (1995) Temporal allele frequency change and estimation of effective population size in populations with overlapping generations. *Genetics*, **139**, 1077–1090.

Jorde P, Ryman N (1996) Demographic genetics of brown trout (*Salmo trutta*) and estimation of effective population size from temporal change in allele frequencies. *Genetics*, **143**, 1369–1381.

Larsen K (1970) Hvor fanges de bornholmske havoredder? In: *Fiskeriundersogelser I 1970 Ved Denmark, Faeroerne Og Gronland* (eds Ursed E, Horsted SA), pp. 10–16. Andr. Fr. Host og Son, Copenhagen.

Larsen PF, Hansen MM, Nielsen EE, Jensen LF, Loeschcke V (2005) Stocking impact and temporal stability of genetic composition in a brackish northern pike population (*Esox lucius* L.), assessed using microsatellite DNA analysis of historical and contemporary samples. *Heredity*, **95**, 136–143.

Leberg P (2005) Genetic approaches for estimating the effective size of populations. *Journal of Wildlife Management*, **69**, 1385–1399.

Lenormand T (2002) Gene flow and the limits to natural selection. *Trends in Ecology & Evolution*, **17**, 183–189.

Lessios HA, Weinberg JR, Starczak VR (1994) Temporal variation in populations of the marine isopod Excirolana: how stable are gene frequencies and morphology? *Evolution*, **48**, 549–563.

Luikart G, Cornuet JM, Allendorf FW (1999) Temporal changes in allele frequencies provide estimates of population bottleneck size. *Conservation Biology*, **13**, 523–530.

Kaeuffer R, Pontiar D, Devillard S, Perrin N (2004) Effective size of two feral domestic cat populations (*Felis catis*): effect of the mating system. *Molecular Ecology*, **13**, 483–490.

Manier MK, Arnold SJ (2005) Population genetic analysis identifies source-sink dynamics for two sympatric garter snake species (*Thamnophis elegans* and *Thamnophis sirtalis*). *Molecular Ecology*, **14**, 3965–3976.

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly (*Ursus arctos*): Implications for conservation. *Proceedings of the National Academy of Sciences, USA*, **100**, 4334–4339.

Mueller LD, Wilcox BA, Ehrlich PE, Heckel DG, Murphy DD (1985) A direct assessment of the role of genetic drift in determining allele frequency variation in populations of *Euphydryas editha*. *Genetics*, **110**, 495–511.

Nei M, Li WH (1973) Linkage disequilibrium in subdivided populations. *Genetics*, **75**, 213–219.

Nei M, Tajima F (1981) Genetic drift and estimation of effective population size. *Genetics*, **98**, 625–640.

Neigel JE (2002) Is F_{ST} obsolete? *Conservation Genetics*, **3**, 167–173.

Nielsen EE, Hansen MM, Loeschcke V (1999) Genetic variation in time and space: microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution*, **53**, 261–268.

Nunney L (1999) The effective size of a hierarchically-structured population. *Evolution*, **53**, 1–10.

Ohta T, Kimura M (1973) A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research*, **22**, 201–204.

Østergaard S, Hansen MM, Loeschcke V, Nielsen EE (2003) Long-term temporal changes of genetic composition in brown trout (*Salmo trutta* L.) populations inhabiting an unstable environment. *Molecular Ecology*, **12**, 3123–3135.

Palsboll PJ, Berube M, Allendorf FW (2007) Identification of management units using population genetic data. *Trends in Ecology & Evolution*, **22**, 11–16.

Potvin C, Bernatchez L (2001) Lacustrine spatial distribution of landlocked Atlantic salmon populations assessed across generations by multilocus individual assignment and mixed-stock analyses. *Molecular Ecology*, **10**, 2375–2388.

Poulsen NA, Nielsen EE, Schierup MH, Loeschcke V, Gronkjaer P (2006) Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Molecular Ecology*, **15**, 321–331.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Raymond M, Rousset F (1995) GENEPOP (3.3): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Ruzzante DE, Taggart CT, Doyle RW, Cook D (2001) Stability in the historical pattern of genetic structure of Newfoundland cod (*Gadus morhua*) despite the catastrophic decline in population size from 1964 to 1994. *Conservation Genetics*, **2**, 257–269.

Saillant E, Gold JR (2006) Population structure and variance effective size of red snapper (*Lutjanus campechanus*) in the north Gulf of Mexico. *Fishery Bulletin*, **104**, 136–148.

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution*, **22**, 25–33.

Slatkin M (2005) Seeing ghosts: the effect of unsampled populations on migration rates estimated for sampled populations. *Molecular Ecology*, **14**, 67–73.

Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. *Biological Conservation*, **87**, 173–180.

Tallmon DA, Luikart G, Beaumont MA (2004) Comparative evaluation of a new effective population size estimator based on approximate Bayesian computation. *Genetics*, **167**, 977–988.

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **8**, 169–179.

Tessier N, Bernatchez L, Wright JM (1997) Population structure and impact of supportive breeding inferred from mitochondrial and microsatellite DNA analyses in land-locked Atlantic salmon *Salmo salar* L. *Molecular Ecology*, **6**, 735–750.

Turner TF, Wares JP, Gold JR (2002) Genetic effective size is three orders of magnitude smaller than adult census size in an abundant, estuarine-dependant marine fish (*Sciaenops ocellatus*). *Genetics*, **162**, 1329–1339.

Vasemagi A, Gross R, Paaver T, Koljinen ML, Nilsson J (2005) Extensive immigration from compensatory hatchery releases into wild Atlantic salmon population in the Baltic Sea: spatio-temporal analysis over 18 years. *Heredity*, **95**, 76–83.

Veliz D, Duchesne P, Bourget E, Bernatchez L (2006) Stable genetic polymorphism in heterogeneous environments: balance between asymmetrical dispersal and selection in the acorn barnacle. *Journal of Evolutionary Biology*, **19**, 589–599.

Vitalis R, Couvet D (2001) Estimation of effective population size and migration rate from one-and two-locus identity measures. *Genetics*, **157**, 911–925.

Vuilleumier S, Possingham HP (2006) Does colonization asymmetry matter in metapopulations? *Proceedings of the Royal Society of London. Series B, Biological Sciences*, **273**, 1637–1642.

Wang J (2001) A pseudo-likelihood method for estimating effective population size from temporally placed samples. *Genetical Research*, **78**, 243–257.

Wang JL (2005) Estimation of effective population sizes from data on genetic markers. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **360**, 1395–1409.

Wang J, Whitlock MC (2003) Estimating effective population size and migration rates from genetic samples over space and time. *Genetics*, **163**, 429–446.

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. *Genetics*, **121**, 379–391.

Waples RS (1990a) Conservation genetics of Pacific salmon III. Estimating effective population size. *Journal of Heredity*, **81**, 277–289.

Waples RS (1990b) Conservation genetics of Pacific Salmon. II. Effective population size and the rate of loss of genetic variability. *Journal of Heredity*, **81**, 267–276.

Waples RS (1991) Genetic methods for estimating the effective population size of cetacean populations. *Report on the International Whaling Commission*, (special issue 13), 279–300.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity*, **89**, 438–450.

Waples RS (2002a) Definition and estimation of effective population size in the conservation of endangered species. In: *Population Viability Analysis* (eds Beissinger SR, McCullough DR), pp. 147–168. University of Chicago Press, Chicago.

Waples RS (2002b) The effective size of fluctuating salmon populations. *Genetics*, **161**, 783–791.

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium data at unlinked gene loci. *Conservation Genetics*, **7**, 167–184.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology*, **15**, 1419–1439.

Waples RS, Smouse PE (1990) Gametic disequilibrium analysis as a means of identifying mixtures of salmon populations. *American Fisheries Society Symposium*, 7, 439–458.

Waples RS, Yokota M (2007) Temporal estimates of effective population size in species with overlapping generations. *Genetics*, **175**, 217–233.

Watts PC, Saccheri IJ, Kemp SJ, Thompson DJ (2007) Effective population sizes and migration rates in fragmented populations of an endangered insect (*Coenagrion mercuriale*: Odonata). *Journal of Animal Ecology*, **76**, 790–800.

Weetman D, Hauser L, Bayes MK, Ellis JR, Shaw PW (2006) Genetic population structure across a range of geographic scales in the commercially exploited marine gastropod *Buccinum undatum*. *Marine Ecology Progress Series*, **317**, 157–169.

Weir BS (1979) Inferences about linkage disequilibrium. *Biometrics*, **35**, 235–254.

Weir BS (1996) *Genetic Data Analysis*. 2nd edn. Sinauer & Associates, Sunderland, Massachusetts.

Whitlock MC, Barton NH (1997) The effective size of a subdivided population. *Genetics*, **146**, 427–441.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: Fst = 1/(4Nm + 1). *Heredity*, **82**, 117–125.

Wilson AJ, Hutchings JA, Ferguson MM (2004) Dispersal in a stream-dwelling salmonid: inferences from tagging and microsatellites studies. *Conservation Genetics*, 5, 25–37.

Wilson G, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, **168**, 1177–1191.

Wright S (1943) Isolation by distance. *Genetics*, **28**, 114–138.

Wright S (1951) The genetic structure of populations. *Annals of Eugenics*, **15**, 323–357.

Dylan Fraser's current research involves the application of genetic and evolutionary principles to biodiversity conservation and fisheries management. Michael Hansen's research focus involves characterizing genetic population structure and the evolution of local adaptations in freshwater and marine fishes. Siri Østergaard and Nathalie Tessier are former graduate students of the Hansen and Bernatchez Laboratories who are still actively involved in biology. Michel Legault is a biologist who studies the ecology of freshwater fishes in Québec, Canada. Louis Bernatchez's major research interests are in understanding patterns and processes of molecular and organismal evolution, as well as their significance to conservation.

Appendix I

Allelic richness (*A*), as well as mean observed and expected heterozygosities (H_O, H_E) for each sample from Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) population, based on seven microsatellite loci (data taken from Tessier & Bernatchez 1999; Fritzner *et al*. 2001; Hansen *et al*. 2002; Østergaard *et al*. 2003). Standard deviations are in parentheses. Geographic locations of populations and sample sizes are found in Figure 1 and Table 1.

Population code	*A*	H_O	H_E	Population code	*A*	H_O	H_E
S. salar means	5.95	0.61	0.62	*S. trutta*-Bornholm means	5.59	0.64	0.65
RS 1970	5.42 (2.38)	0.60 (0.07)	0.62 (0.11)	VE 1950	7.14 (2.91)	0.64 (0.17)	0.68 (0.16)
RS 1994	6.14 (2.75)	0.68 (0.12)	0.68 (0.09)	VE 1992	5.14 (2.19)	0.69 (0.15)	0.67 (0.14)
ASH 1978	7.00 (3.46)	0.64 (0.22)	0.64 (0.22)	VE 1997	5.86 (2.79)	0.62 (0.14)	0.67 (0.12)
ASH 1994	6.86 (3.76)	0.61 (0.20)	0.64 (0.17)	BL 1944	5.71 (2.36)	0.62 (0.33)	0.59 (0.27)
OUA 1980	6.71 (3.49)	0.63 (0.16)	0.68 (0.12)	BL 1950	7.00 (3.11)	0.68 (0.17)	0.68 (0.14)
OUA 1994	6.57 (3.42)	0.65 (0.17)	0.68 (0.13)	BL 1997	4.86 (2.79)	0.63 (0.18)	0.67 (0.14)
MET 1981	4.29 (2.71)	0.54 (0.30)	0.51 (0.26)	DO 1966	5.14 (2.34)	0.73 (0.15)	0.68 (0.13)
MET 1994	4.57 (2.06)	0.50 (0.24)	0.51 (0.23)	DO 1992	5.00 (2.58)	0.72 (0.22)	0.69 (0.14)
				DO 1997	4.14 (2.04)	0.58 (0.18)	0.58 (0.20)
Other *S. trutta* means	5.81	0.64	0.66	TE 1944	6.00 (1.63)	0.68 (0.12)	0.68 (0.11)
MOS 1998	5.43 (2.23)	0.66 (0.09)	0.66 (0.10)	TE 1951	6.86 (2.12)	0.63 (0.22)	0.65 (0.23)
VEJ 1910	6.14 (3.13)	0.58 (0.18)	0.68 (0.16)	TE 1992	4.86 (2.48)	0.64 (0.19)	0.62 (0.15)
VEJ 1998	6.86 (2.54)	0.69 (0.12)	0.69 (0.14)	TE 1997	6.14 (3.67)	0.54 (0.20)	0.61 (0.20)
KAR 1912	6.43 (2.76)	0.59 (0.14)	0.64 (0.15)	GR 1997	4.57 (1.62)	0.62 (0.14)	0.62 (0.14)
KAR 1951	6.00 (1.63)	0.62 (0.14)	0.66 (0.15)	LAE 1997	5.00 (2.16)	0.67 (0.12)	0.63 (0.15)
KAR 1993	5.71 (2.56)	0.68 (0.12)	0.66 (0.11)	BA 1997	6.00 (3.74)	0.59 (0.22)	0.65 (0.14)
KAR 1996	5.43 (2.07)	0.67 (0.13)	0.67 (0.11)				
KOV 1953	5.86 (2.67)	0.63 (0.10)	0.64 (0.09)				
KOV 1996	5.29 (2.63)	0.66 (0.14)	0.65 (0.12)				
ODR 1998	5.14 (2.19)	0.58 (0.21)	0.62 (0.18)				
KOL 1998	5.57 (2.70)	0.63 (0.13)	0.66 (0.15)				

Appendix II

We applied three contrasting metapopulation models to explore different scenarios for estimating the effective metapopulation size ($metaN_e$) of Bornholm trout populations. First, we applied Wright's (1943) finite island model:

$$metaN_e = N_T/(1 - F_{ST}),$$

where $N_T = nN$ (the number of subpopulations, which was taken as 4, 8, and 12, multiplied by N_e of each subpopulation, which for simplicity we took as the mean N_e between our sampled rivers from Methods 1–3), and F_{ST} is the global degree of genetic differentiation between all subpopulations within the metapopulation. Global θ_{ST} in Bornholm, an analogue of F_{ST}, was 0.086 circa 1950 and 0.031 circa 1997, so we took the mean of these two values ($\approx$ 0.0586) for our calculations. This model makes the most simplistic assumptions about metapopulation structure, for instance, that subpopulations have equal N, that they cannot go extinct, that they receive the same fraction of migrants drawn randomly from the migrant pool, and that they have random mating. Under Wright's model, any population subdivision (F_{ST} or $\theta_{ST} > 0$) results in $metaN_e$ being greater than the sum of subpopulation N_e's (Waples 2002a).

Second, we applied Nunney's (1999) interdemic genetic drift model:

$$metaN_e = \frac{N_T}{(1 + F_{IS})(1 + F_{ST}) - 2F_{IS}F_{ST}},$$

where F_{ST} is the global F_{ST} between subpopulations ($\approx$ 0.0586), and F_{IS} is the global inbreeding coefficient (Wright 1943) within subpopulations (calculated as 0.0155 for Bornholm). This model considers more realistically that random differences in reproductive success may build up in different subpopulations. These can affect a subpopulation's productivity and thus its contribution to the migrant pool, which usually leads to a lower $metaN_e$ than the sum of individual subpopulation N_e's (Nunney 1999).

Third, we considered the extinction-recolonization model of Whitlock & Barton (1997; equation 23, simplified):

$$metaN_e = \frac{n}{4(m + e)F_{ST}},$$

where m is the rate of migration between subpopulations and e is the subpopulation extinction rate. This model assumes that the variance in productivity between subpopulations is primarily due to extinction and recolonization that occur within the metapopulation, a very probable feature of Bornholm trout population structure (Østergaard *et al*. 2003). Under this model, $metaN_e$ is strongly influenced by the number of subpopulations (n) comprising the metapopulation. We considered three scenarios of migration that typified observed m values on Bornholm (e.g. m = 0.05, 0.10, 0.15; Tables 3, 5), as well as two moderate extinction rates (e = 0.1, 0.2), given that extinction–recolonization was observed in some rivers between the 1950s and 1990s.